Spirocaracolitones, CD-Spiro Triterpenoids from *Ruptiliocarpon caracolito*

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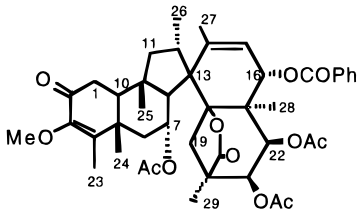
Six CD-spiro-triterpenoids have been isolated and identified from the methylene chloride soluble extracts of the bark of *Ruptiliocarpon caracolito*. The structures of these compounds, named spirocaracolitones A−F, were determined by a combination of X-ray and high field NMR studies. The spirocaracolitones represent the first examples of a class of triterpenes which have a spiro system connecting rings C and D; they also carry an unusual 12-α-methyl group. It is postulated that these compounds are derived biogenetically from a friedelin derivative. Initial biological screening revealed up to 80% decreases in the rate of growth of the larvae of the European corn borer when these compounds were administered at the rate of 50 ppm in the diet.

Introduction

Several years ago we initiated a program aimed at the discovery of new insecticides from plants native to Central America.[1] Lyophilized ethanol extracts of various parts of 25 species of the Meliaceae family, tropical hardwood trees which are native to this region, were screened against the European corn borer (*Ostrinia nubilalis*). The bark extract of *Ruptiliocarpon caracolito* was found to be the most active in our second instar bioassay. This tree species was originally thought to belong to the Meliaceae family because of its similarities to the wood and floral parts of the Trichilia genus. Recent studies suggest that *R. caracolito* does not belong to the Meliaceae but to a unique American species of the family Lepidobotryaceae with close affinity to the monotypic African genus *Lepidobotrys*. The discovery of this species in Costa Rica was such an unusual find that it was cited recently in the National Geographic as an "unfamiliar tree" belonging to a family of trees previously known only in Africa.[2]

Earlier work on the bark of *R. caracolito* resulted in the isolation of a unique CD-spirotrieterpenoid, compound **1**.[3] To the best of our knowledge neither the CD spiro arrangement nor the 12-α-methyl group present in **1** have been previously observed in triterpenoids. We have suggested that **1** was biogenetically derived from a friedelin precursor. Since our initial report we have isolated and identified five additional compounds which are closely related to structure 1.



1, spirocaracolitone A

Isolation and Structure Elucidation

R. caracolito is endemic to Costa Rica and was collected near Golfito in a humid lowland tropical rain forest area on the Osa peninsula. The ethanol extract of the bark of *R. caracolito* reduced the relative growth (percent of control) of the second instar larvae of the European corn borer by greater than 80% when incorporated into the diets at 50 ppm. This represented the greatest growth reduction of any of the extracts screened.

The freeze-dried ethanol extract was separated into hexane, dichloromethane, and water soluble portions. When these portions were screened for activity, the dichloromethane soluble fraction was found to contain most of the activity and was therefore subjected to silica gel chromatography. A major component of the most active fractions thus obtained was subsequently purified by preparative HPLC to yield a white crystalline compound, $C_{44}H_{54}O_{12}$, mp 215−218 °C, in about 0.01% yield from dried bark. The structure of this compound, named spirocaracolitone, was established as **1** by single crystal X-ray structure determination (Figure 1).[3] Subsequent repetitive fractionation of the other components of the most active fractions, using a recycling HPLC equipped with a reverse phase column, enabled us to isolate and identify five additional CD-spirotriterpenoids, each in approximately 0.05−0.008% yield. For simplicity and consistency we would like to rename the first identified compound as spirocaracolitone A, and the newly isolated compounds as spirocaracolitones B−F, **2**−**6**, respectively). This renaming seems reasonable since the first identified compound became one of a series. Continuing investigations have resulted in the isolation of at least four additional spirotriterpenoids.[4] The structures of these additional compounds have only been tentatively assigned and therefore will be discussed in a later publication describing the insecticidal structure−activity relationships of all the isomers.

The structures of spirocaracolitones B−F were determined by a combination of proton and carbon NMR spectroscopy and X-ray structure determinations carried out on spirocaracolitones B (**2**) and E (**5**). These com-

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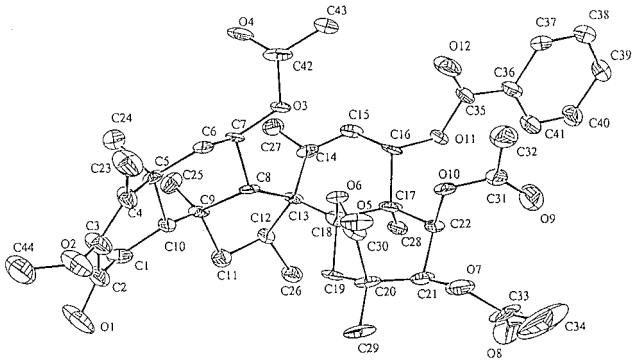
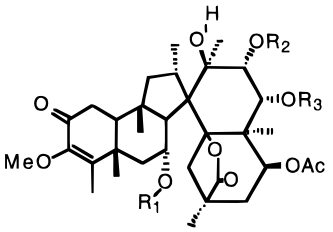
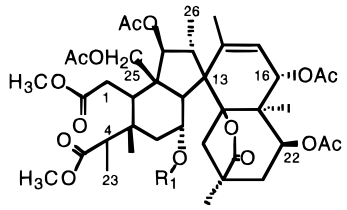


Figure 1. ORTEP of spirocaracolitone A.



2, spirocaracolitone B: $R_1 = R_2 = R_3 = Ac$
3, spirocaracolitone C: $R_1 = H$; $R_2 = Ac$; $R_3 = COPh$
4, spirocaracolitone D: $R_1 = Ac$; $R_2 = H$; $R_3 = COPh$



5, spirocaracolitone E: $R = COC(CH_3)=CHCH_3$
6, spirocaracolitone F: R = COPh

pounds are related to the friedelin family of triterenes and thus, though not proven, it is most likely that the absolute stereochemistry is as shown. Once the structure of spirocaracolitone A (**1**) had been determined by single crystal X-ray analysis (Figure 1), the assignments of most of the proton and carbon resonances were made. It was expected that a thorough understanding of the spectra of **1** would aid in the structure determination of the remaining spirotriterpenoids. The infrared spectra of **1** showed carbonyl stretching frequencies at 1780, 1750, 1722, and 1674 cm^{-1}, characteristic of the γ-lactone, acetate, benzoate and α,β-unsaturated ketone moieties.

Six isolated proton spin systems were identified in the spectrum of **1** in addition to the various quaternary methyls, three acetates, and one benzoate group. The system $H1_{ax}$, $H1_{eq}$, and H10 showed three sets of peaks at 2.59, 2.34, and 1.83 ppm, respectively, with coupling constants of 17.2 (gem.), 14.3 (ax−ax) and 3.4 (ax−eq) Hz. The carbon resonances at 37.2 and 55.9 ppm were assigned to C1 and C10, respectively, using the HMQC spectrum.

The doublet of triplets at 5.31 ppm, which had J values of 3.2 and 10.9 Hz due to an ax−eq and two ax−ax interactions, was assigned to $H7_{ax}$. The signal for $H6_{eq}$ occurred as a doublet of doublets, $J = 3.2$ (ax−eq) and 12.2 (gem.) Hz, at 2.11 ppm. The position of the signals for the remaining two hydrogens in this spin system, $H6_{ax}$

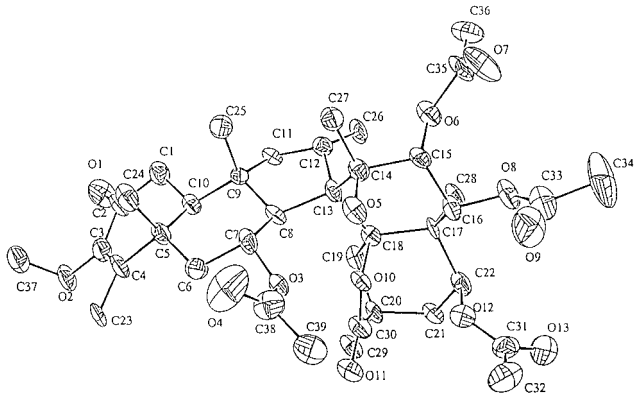


Figure 2. ORTEP of spirocaracolitone B.

(1.45−1.54 ppm) and H8 (centered near 2.30 ppm) were assigned using a COSY spectrum.

The narrow multiplet at 6.02 ppm was assigned to H16. In the COSY spectrum this resonance showed coupling to a broad singlet at 5.45 ppm (H15) and to the allylic methyl group attached to C14. The carbon resonances for C14 (136.6 ppm), C15 (125.5 ppm), C16 (69.9 ppm), and the allylic methyl group (23.9 ppm) were assigned based inspection of the HMQC spectrum.

The final two isolated spin systems were due to the C19 methylene group, a set of doublets at 2.43 and 2.62 ppm with a 12.7 Hz geminal coupling constant, and the cis arranged hydrogens at C21 and C22 (doublets at 5.20 and 5.23 ppm, $J = 4.4$ Hz). It was not possible to make the individual assignments in the latter case. Most of the remaining carbon resonances of **1**, and eventually of all of the other spirotriterpenoids, were confirmed using HMQC and HMBC spectra. These are listed with the assignments, where firm, in the Experimental Section. The spiro carbon, C13, occurred at 57.6 ppm.

The structure of spirocaracolitone B (**2**), $C_{39}H_{54}O_{13}$, mp 240 °C dec., was secured by single crystal X-ray structure determination (Figure 2). It differed from the first spirotriterpenoid in three ways: (i) the C14−C15 double bond was dihydroxylated and the secondary hydroxyl group at C15 further acetylated; (ii) the benzoate at C16 was replaced by an acetate; and (iii) the acetate at C21 was replaced by a hydrogen atom.

The loss of the acetoxy group at C21 created an ABX system in **2** replacing the AB system observed for the two hydrogens on C21 and C22 in **1**. The C22 hydrogen at 4.89 ppm coupled to the two hydrogens on C22 (1.78 and 2.06 ppm). The changes in ring D in going from **1** to **2** gave rise to two doublets at 5.95 and 5.18 ppm due to H15 and H16. The lower field signal was assigned to H15 because the adjacent carbons carried oxygen functionalities. The observed coupling constant of 3.7 Hz agreed with an eq−ax arrangement between these hydrogens. These conclusions are in agreement with the X-ray data which showed the free hydroxyl group at C14 (IR peak at 3531 cm^{-1}) in an axial position. The trans relationship between the C14 and C15 oxygen atoms is consistent with an epoxidation of the C14 double bond followed by a trans diaxial ring opening in accordance with the Furst−Plattner rule.[5] The partial structure of spirocaracolitone B, consistent with these data, is shown in Figure 3.

(5) Eliel, E. L.; Allinger, N. L.; Angyal, S. J.; Morrison, G. A. *Conformationa Analysis*; John Wiley and Sons: New York, 1965.

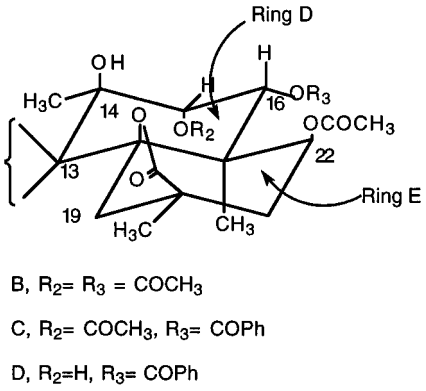


Figure 3. Partial structures of spirocaracollitones B−D.

Two isomeric compounds ($C_{42}H_{54}O_{12}$), closely related to spirocaracolitone B were subsequently isolated. These were designated spirocaracolitone C, **3**, mp 228 °C dec. and spirocaracolitone D, **4**, mp 251−256 °C. A common difference between these compounds and spirocaracolitone B was the replacement of the acetyl group at C16 with a benzoate group.

For spirocaracolitone C, it was clear that the free hydroxyl group was located at C7. In spirocaracolitone B the signal for H7 appeared as a doublet of triplets at 5.27 ppm with the expected 3.7 Hz ax−eq and 10.4 Hz ax−ax coupling constants (Table 1). In spirocaracolitone C this doublet of triplets, with similar coupling constants, was observed at 4.13 ppm. This chemical shift change was consistent with the conversion: CHOAc → CHOH. The pair of doublets at 5.45 and 6.40 ppm (J = 3.3 Hz) were assigned to the CHOAc and CHOBz at C15 and C16, respectively.

In the structure assigned for spirocaracolitone D (**4**), the hydroxyl group at C7 is acetylated and H7 observed as a dt (J = 3.1, 10.4 Hz) at 5.43 ppm. The C16-hydroxyl group carries a benzoyl group. The hydrogen remaining on C16 appeared as a doublet (J = 4.8 Hz) at 6.01 ppm; H15 is observed as a broad singlet at 4.53 ppm.

The final two spirotriterpenoids thus far identified have been designated spirocaracolitone E ($C_{45}H_{62}O_{16}$) and F ($C_{47}H_{60}O_{16}$). The infrared and proton NMR spectra of these compounds differed significantly from the other spirotriterpenoids described earlier. Most importantly, they lacked the 1674 cm^{-1} absorption in the infrared due to the α,β-unsaturated ketone system in ring A. Both compounds retained the bridging γ-lactone feature, in ring E as indicated by the strong band at 1783 cm^{-1}. In spirocaracolitone E (**5**) the carbonyl frequency at 1697 cm^{-1} was consistent with an α,β-unsaturated ester. This was confirmed as a tigloyl group by the ^{1}H NMR which showed two methyl signals at 1.81 and 1.91 ppm and an olefinic hydrogen at 7.07 ppm which coupled to each of these methyl groups.

For spirocaracolitone F (**6**) the unsaturated ester at 1720 cm^{-1} was due to the benzoate group. Interestingly, the ortho resonances on the benzene ring absorbed at a lower field, 8.26 ppm, than the more normal position of such hydrogens as illustrated in the spectra of compounds

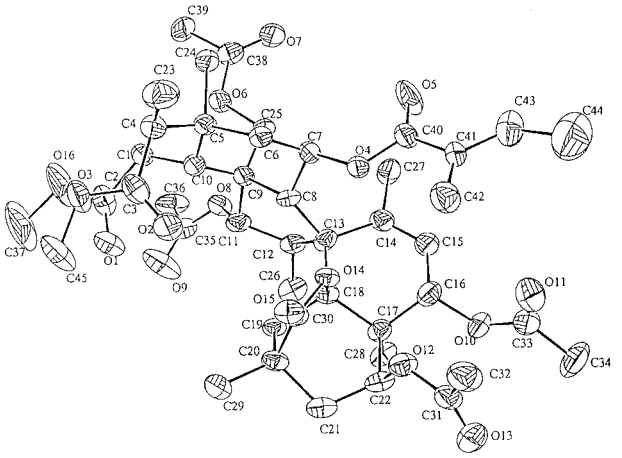


Figure 4. ORTEP of spirocaracolitone E.

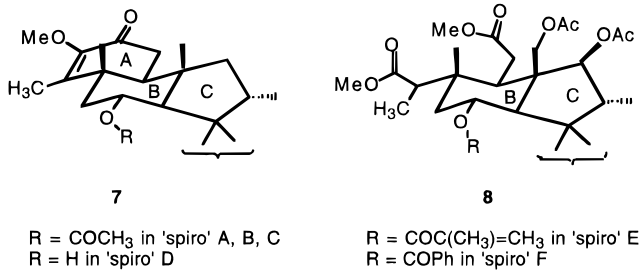


Figure 5. The ABC ring system of spirocaracolitones A−D, and the B and C ring of spirocaracolitones E and F.

1, **3**, and **4**. Both spirocaracolitone E and F showed four acetate and two methyl ester groups.

The presence of two ester functions adjacent to ring B was proven by an X-ray structure determination of spirocaracolitone E (Figure 4). This suggested that these functionalities were the result of oxidative cleavage of the A ring. Also observed from the X-ray data was that hydroxylation and subsequent acetylation had taken place at C11 and on the C25 methyl group. Ring D was unsaturated as in spirocaracolitone A (**1**), with ring F having the same pattern as found in **2**−**4**. The changes are shown in the partial structures **7** → **8** (Figure 5).

These features were consistent with the proton NMR spectrum of spirocaracolitone E. The common spin systems seen earlier in the other spirotriterpenoids, such as the C19 methylene group, the three-nuclei spin system due to the hydrogens on C21 and C22, and the allylic system of ring C, are reported for comparison in Table 2.

The acetate function at C11 simplified the proton NMR of that part of spirocaracolitone E compared to that of the spirotriterpenoids discussed earlier. The doublets observed at 5.22 (J = 13 Hz) and 5.26 ppm (J = 12.1 Hz) in spirocaracolitone E and spirocaracolitone F, respectively, were assigned to H11. The large coupling constants were in agreement with the trans arrangement between H11 and H12. The CH_2OAc group attached to C9 gave rise to isolated AB systems at 4.60 and 4.91 ppm (J_{AB} = 12.9 Hz) for spirocaracolitone E, and at

Table 1. A Comparison of ^{1}H NMR Shifts (ppm) of Selected Protons in Spirocaracolitones B−D

	spirocaracolitone B	spirocaracolitone C	spirocaracolitone D
H7	5.27 (dt, J = 3.7, 10.4 Hz)	4.13 (dt, J = 3.2, 10.4 Hz)	5.43 (dt, J = 3.1, 10.4 Hz)
H15	5.45 (d, J = 3.7 Hz)	5.45 (d, J = 3.3 Hz)	4.53 (brs)
H16	5.18 (d, J = 3.7 Hz)	6.40 (d, J = 3.3 Hz)	6.01 (d, J = 4.8 Hz)

Table 2. ^{1}H NMR Shifts (ppm) of Selected Protons in Spirocaracolitones E and F

	spirocaracolitone E	spirocaracolitone F
H11	5.22 (d, J = 13.0 Hz)	5.26 (d, J = 12.1 Hz)
H12	2.79 (m)	2.82 (m)
H19	2.37 (m)	2.41 (m)
H21	1.93 (m)	1.89−1.95 (m)
H22	4.90 (m)	4.88 (dd, J = 1.3, 4.4 Hz)

4.51 and 4.91 ppm (J_{AB} = 12.8 Hz) for spirocaracolitone F. The C7 hydrogen appeared as the expected doublet of triplet pattern at 5.58 ppm for C7 carrying the tigloyl group in spirocaracolitone E and at 5.78 ppm in spirocaracolitone F where it carries a benzoyl group. These chemical shifts can be compared to the 5.3−5.5 ppm shift which was observed when the C7 hydroxyl group was acetylated as in spirocaracolitone A, C, and D. Molecular models indicate that the ortho hydrogens of the benzoyl group in F are close to the bridging lactone which may account for the unusually low chemical shift of these hydrogens.

Biological Activity of the Spirocaracolitones

The effect of these compounds on the life cycle of the European corn borer has been investigated. Figure 6 shows the variations in growth of 30 test larvae fed a diet containing 50 ppm of the spirotriterpenoids. Spirocaracolitone F had the greatest growth reducing effect, followed by spirocaracolitone E and A. Relatively little activity against the corn borer larvae was noted for spirocaracolitones B, C, and D. The same order of activity was noted for these compounds on other biological parameters such as duration of the pupal stage, % pupation, days to adult emergence, and the overall mortality rates. A detailed discussion of these data and the response of these compounds to other insect species will be published elsewhere.

Possible Biogenetic Pathways to the Spirocaracolitone Ring System

As was pointed out earlier, these compounds have structural features which have not been observed previously in pentacyclic triterpenoids. There appear to be no examples of such compounds having either a methyl group at C12, or a spiro CD ring junction. We surmise that these two features are biogenetically interdependent. These highly oxygenated compounds also display a somewhat unusual oxidation pattern in ring A which has been seen in 3-hydroxyfriedel-3-en-2-one, isolated from the bark of *Quercus suber*[6] and in 3-hydroxy-2-oxofriedel-3-en-29-oic acid.[7] The remaining oxygen functions, such as the C18−C30 lactone and the acetates, benzoates, or tiglate groups at various positions, have been individually reported previously, but not in combination.[8]

The spirocaracolitones appear to be highly oxidized friedelin derivatives. A friedelin derivative bearing an α-hydroxyl group at C12 could be a suitable precursor for the formation of a C12 carbocation intermediate [**10**, R = (+), Scheme 1]. Migration of the C27 methyl group from C13 to C12 would generate a cationic center at C13 (**11**) which would allow for migration of the C8−C14 bond. This mechanism would create both required features: the CD spiro ring system and the C12 methyl group. Loss of a proton from C15 would generate the C14 double bond seen in structure **12**. Alternatively, a proton could be lost from the newly generated spiro system creating a C13−C18 double bond which upon epoxidation would lead to **13**. Finally, acid-catalyzed epoxide opening with concomitant migration of the C8−C14 bond would generate **12** (R = H). The timing of the introduction of the various oxygen functions relative to the creation of the spiro system is not known.

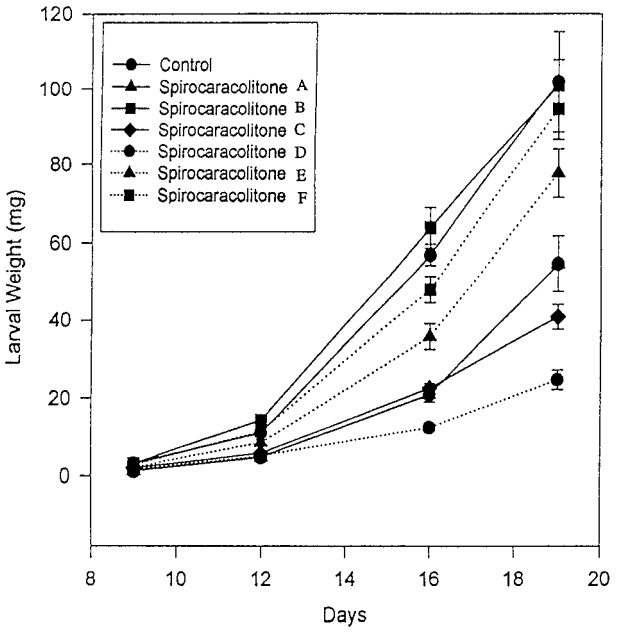
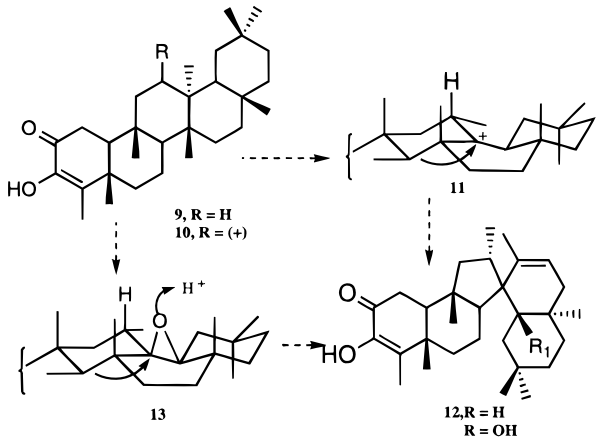


Figure 6. Mean larval weights of the European corn borer exposed to 50 ppm of spirocaraclitones in diet.

Scheme 1. A Postulated Biogenetic Origin of the CD-Spiro Ring System of the Spirocaracolitones



Experimental Section

General Methods and Materials. Melting points were determined by the using Thomas-Hoover Capillary melting point apparatus and are uncorrected. Chemical ionization mass spectra were obtained using isooctane as carrier on a VG 7070E or a Kratos concept 2H instrument. IR spectra were recorded in CH_2Cl_2 solution employing a Bomem−Michelson MB-100 FT/IR spectrophotometer. NMR spectra were obtained in $CDCl_3$ on a Bruker AMX-500 NMR spectrometer. Optical rotation were determined using a Perkin-Elmer polarimeter (Model 241) set on the sodium D line. Solvents for extractions and chromatographic purifications were routinely distilled before use. The 95% ethanol used for the initial bark extraction was used as supplied. Column chromatographic

(6) Patra, A.; Chaudri, S. K. *Mag. Reson. Chem. 25*, 95.
(7) Coto, A. B.; Mascarenko, Y. P.; Silva, G. D. F.; deSouza, J. D. *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.* **1990**, *46*, 328.
(8) Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **1987**, 421−422.

purifications were performed with silica gel 270−400 Mesh. Analytical HPLC work was performed on a Varian 9012 HPLC equipped with a Varian Variable wavelength UV-vis detector set at 254 nm and a 5 mm Techsphere ODS column (4.6 mm × 25 cm). Preparative HPLC work was performed on a LC-908 JAIGEL Recycling HPLC equipped with a fixed wavelength UV detector (254 nm) and a 15 mm reverse phase column (ODS-S-343-15). HPLC grade acetonitrile (Omnisolv, BDH) was filtered through Millipore filters before use. Water for the HPLC work was obtained from a Millipore water filtration system.

Isolation of Crude Spirocaracolitone Fraction. The bark of *R. caracolito* was collected in Golfito, Costa Rica and was preserved in 1 L nalgene bottles containing 95% ethanol for transport into Canada. In the laboratory the ethanol was decanted and put aside while the bark was allowed to dry in the fumehood overnight. When the bark was dry it was ground into sawdust-sized particles using a Wiley mill. The ethanol previously decanted from the bark was then added back to the ground sample. Often additional ethanol was added to ensure that the sample was completely immersed in solvent. Extraction of the bark with 95% ethanol was performed three times at room temperature for a 24 h period. The combined extracts were evaporated using a rotary evaporator and freeze dried, yielding a gummy solid.

The extract was then reconstituted in a 1:1 ethanol/water solution and extracted four times with hexane. Most of the ethanol of the remaining ethanol/water layer was then removed in vacuo so that the layer could be further extracted with CH_2Cl_2 (four times) without formation of an emulsion. The CH_2Cl_2 layers were combined and condensed. Flash chromatography of the CH_2Cl_2 soluble extract, eluted with CH_2Cl_2/ethyl acetate in a gradient of 0 to 100%, resulted in the collection of a fraction containing the spirocaracolitones.

Isolation of Purified Spirocaracolitones. The procedure given above is an improvement over that reported for the isolation of the first spirocaracolitone.[3]

Flash chromatography of the spirocaracolitone-containing fraction using acetone/CH_2Cl_2 elution of 0 to 25% resulted in the partial subdivision of these compounds. The purity of the fractions were monitored using an analytical HPLC equipped with a reverse phase column and eluted isocratically, at a rate of 0.9 mL/min, with 40% water in acetonitrile. Isolation of these six spirotriterpenoids was accomplished using a recycling HPLC equipped with a semipreparative reverse phase column eluted, at a rate of 5 mL/min, with either 20% or 40% water in acetonitrile. Often 3−6 cycles were required before a suitable separation of the components was obtained. Spirocaracolitones B−F were isolated in yields of 0.05 to 0.08% per dry weight of bark.

X-ray quality crystals were obtained via crystallization of spirocaracolitones from methanol which contained a small amount of water.

Spirocaracolitone A (1): mp 215−218 °C; $[\alpha]^{25}_D = +39.3$ (*c* 0.006, CH_2Cl_2); IR 1780, 1750, 1722, 1674 cm^{-1}; ^{1}H NMR[6] δ 2.34 (dd, 1 H, J = 3.4, 17.2 Hz, $H1_{eq}$), 2.59 (dd, 1 H, J = 14.3, 17.2 Hz, $H1_{ax}$), 1.45−1.54 (m, 1 H, $H6_{ax}$), 2.11 (dd, 1 H, J = 3.2, 12.2 Hz, $H6_{eq}$), 5.31 (dt, 1 H, J = 3.2, 10.9 Hz, H7), 2.30 (m, 1 H, H8), 1.80−1.85 (m, 1 H, H10), 1.35−1.55 (m, 2 H, H11), 2.92 (m, 1 H, H12), 5.45 (brs, 1 H, H15), 6.02 (m, 1 H, H16), 2.43 (d, 1 H, J = 12.9 Hz, H19), 2.62 (d, 1 H, J = 12.5 Hz, H19), 5.20 and 5.23 (d, 2 H, J = 4.4 Hz, H21 and H22), 1.83 (s, 3 H, C23), 1.18, 1.23, 1.47 (3 s, C24, C25, and/or C28), 1.23 (d, 3 H, J = 7.5 Hz, C26), 1.87 (brs, 3 H, C27), 1.22 (s, 3 H, C29), 3.61 (s, 3 H, C3-OMe), 1.91, 1.94, 2.28 (3 s, 3 acetyl methyls), benzoyl group: 7.89 (m, 2 H, H2′), 7.39 (m, 2 H, H3′), 7.52 (m, 1 H, H4′); ^{13}C NMR δ 37.2 (C1), 193.9 (C2), 147.7 (C3), 155.1 (C4), 44.2 and 41.9 (C5 and C9), 46.0 (C6), 66.8 (C7), 61.6 (C8), 55.9 (C10), 50.5 (C11), 40.0 (C12), 57.6 (C13), 136.6 (C14), 125.5 (C15), 69.9 (C16), 47.2 (C17), 90.5 (C18), 42.4 (C19), 43.9 (C20), 72.7 (C21), 72.1 (C22), 11.3 (C23), 18.5, 19.4, 21.4 (C24, C25, C28), 17.9 (C26), 23.9 (C27), 16.3 (C29), 175.9 (C30), 20.2, 20.4, 21.3 (3 acetyl methyls), 59.7 (C3-OMe), 169.8, 170.1, 171.6 (acetyl carbonyls), benzoate group: 129.8 (C1′), 129.4 (C2′), 128.4 (C3′), 133.2 (C4′) 166.0 (C=O); MS (FAB) m/z 775 $(MH)^+$ (20.4), 715 (2.1), 593 (4.4), 533 (3.0), 473 (2.1), 105 (100).

Spirocaracolitone B (2): mp 240 °C dec; $[\alpha]^{25}_D = -22.2$ (*c* 0.005, CH_2Cl_2); IR 3531, 2940, 1787, 1741, 1674 cm^{-1}; ^{1}H NMR δ 2.34 (m, 1 H, $H1_{eq}$), 2.56 (dd, 1 H, J = 14.2, 17.1 Hz, $H1_{ax}$), 1.18 and 2.35 (m, 2 H, H6), 5.27 (dt, 1 H, J = 3.7, 10.4 Hz, H7), 2.05 (m, 1 H, H8), 1.74 (dd, 1 H, J = 3.4, 14.2 Hz, H10), 1.28 (m, 1 H, $H11_{eq}$), 1.52 (dd, 1 H, J = 5.7, 12.4 Hz, $H11_{ax}$), 2.56 (m, 1 H, H12), 5.95 (d, 1 H, J = 3.7 Hz, H15), 5.18 (d, 1 H, J = 3.7 Hz, H16), 2.32 (m, 1 H, H19), 2.49 (d, 1 H, J = 12.7 Hz, H19), 1.78 (d, 1 H, J = 14.4 Hz, H21), 2.06 (m, 1 H, H21), 4.89 (dd, 1 H, J = 1.3, 4.4 Hz, H22), 1.82 (s, 3 H, H23), 1.35 (d, 3 H, J = 8.3 Hz, H26), 1.13, 1.22, 1.35, 1.55, 1.92, 1.96, 2.08, 2.14, 2.15 (9 s, 9 × 3 H, consisting of 5 quaternary methyls and 4 acetyl methyls), 3.57 (s, 1 H, C3-OMe), 4.13 (brs, 1 H, C14-OH); ^{13}C NMR δ 37.0 (C1), 194.0 (C2), 147.8 (C3), 154.7 (C4), 43.6 (C5), 47.1 (C6), 70.2 (C7), 65.0 (C8), 43.6 (C9), 55.1 (C10), 52.8 (C11), 37.8 (C12), 66.2 (C13), 76.7 (C14), 65.4 and 77.5 (C15 and C16), 45.3 (C17), 92.9 (C18), 40.4 (C19), 40.9 (C20), 37.7 (C21), 74.3 (C22), 11.2 (C23), 26.8 (C26), 176.3 (C30), 19.9, 20.1, 20.3, 20.4, 20.6, 20.8, 21.1, 21.4 (5 quat. methyls and 4 acetyl methyls), 59.7 (C3-OMe), 169.2, 169.5, 170.1, 170.2 (4 C=O); MS (CI/ISO) m/z 731 $[MH]^+$ (31.1), 671 (25.5), 611 (40.8), 551 (43.7), 491 (27.5), 109 (100).

Spirocaracolitone C (3): mp 228 °C dec; $[\alpha]^{25}_D = -20.4$ (*c* 0.005, CH_2Cl_2); IR 3539, 3301, 2938, 1775, 1738, 1675 cm^{-1}; ^{1}H NMR δ 2.33 (dd, 1 H, J = 3.5, 17.4, Hz, $H1_{eq}$), 2.53 (dd, 1 H, J = 14.2, 17.2 Hz, $H1_{ax}$), 2.45 (dd, 1 H, J = 3.0, 12.9 Hz, $H6_{eq}$), 1.14 (m, 1 H, $H6_{ax}$), 4.13 (dt, 1 H, J = 3.2, 10.4 Hz, H7), 1.83 (d, 1 H, J = 7.8, Hz, H8), 1.67 (dd, 1 H, J = 3.3, 14.3 Hz, H10), 1.50 (dd, 1 H, J = 5.8, 12.0 Hz, $H11_{eq}$), 1.29 (m, 1 H, $H11_{ax}$), 2.61 (m, 1 H, H12), 5.45 (d, 1 H, J = 3.3 Hz, H15), 6.40 (d, 1 H, J = 3.3 Hz, H16), 2.33 (m, 1 H, H19), 2.58 (d, 1 H, J = 12.8 Hz, H19), 2.11 (dd, 1 H, J = 4.5, 19.5 Hz, $H21_{eq}$), 1.81 (m, 1 H, $H21_{ax}$), 4.92 (d, 1 H, J = 4.4 Hz, H22), 1.86 (s, 3 H, H23), (d, 3 H, J = 7.2 Hz, H26), 1.09, 1.17, 1.20, 1.45, 1.70 (5 s, 5 quat. methyls), 1.97, 2.06 (2, s, 2 acetyl methyls), 3.58 (s, 3 H, C3-OMe), benzoyl group: 7.83 (m, 2 H, H2′), 7.35 (m, 2 H, H3′), 7.48 (m, 1 H, H4′); ^{13}C NMR δ 37.0 (C1), 193.9 (C2), 147.9 (C3), 154.4 (C4), 43.6 and 45.6 (C5 and C17), 51.0 (C6), 67.4 (C7), 67.8 (C8), 41.0 and 40.8 (C9 and C20), 55.4 (C10), 52.9 (C11), 38.6 (C12), 66.2 (C13), 76.5 (C14), 66.6 and 77.5 (C15 and C16), 93.3 (C18), 41.6 (C19), 37.3 (C21), 74.1 (C22), 11.2 (C23), 21.0 (C26), 178.6 (C30), 19.6, 20.0, 20.2, 20.5, 28.3 (5 methyl peaks, C24, C25, C27, C28, or C29), 59.6 (C3-OMe), 21.0 (Me-acetate), 169.0 and 170.3 (C=O); benzoate group: 130.0 (C1′), 129.4 (C2′), 128.3 (C3′), 132.9 (C4′), 165.0 (C=O), MS(FAB) m/z 1502 (9.0), 751 $[M + H]^+$ (2.4), 7.33 (5.1), 691 (1.0), 631 (1.3), 611 (3.8), 551 (6.3), 509 (7.9), 105 (100).

Spirocaracolitone D (4): mp 251−256 °C; $[\alpha]^{25}_D = -21.7$ (*c* 0.002, CH_2Cl_2); IR 2968, 1781, 1731, 1674 cm^{-1}; ^{1}H NMR δ 2.35 (dd, 1 H, J = 3.5, 17.3 Hz, $H1_{eq}$), 2.55 (dd, 1 H, J = 14.3, 17.2 Hz, $H1_{ax}$), 1.24 and 2.24 (m, 2 H, H6), 5.43 (dt, 1 H, J = 3.1, 10.4 Hz, H7), 1.98 (d, 1 H, J = 10.4 Hz, H8), 1.70 (dd, 1 H, J = 3.4, 14.1 Hz, H10), 1.24 and 1.52 (m, 2 H, H11), 2.79 (m, 1 H, H12), 4.53 (brs, 1 H, H15), 6.01 (d, 1 H, J = 4.8 Hz, H16), 2.27 (m, 1 H, H19), 2.49 (d, 1 H, J = 12.8 Hz, H19), 1.86 (d, 1 H, J = 14.9 Hz, H21), 2.00 (m, 1 H, H21), 4.92 (dd, 1 H, J = 1.5, 4.4 Hz, H22), 1.82 (s, 3 H, H23), 1.31 (d, 3 H, J = 7.3 Hz, H26), 1.65 and 3.31 (brs, 2 × 1 H, C14OH and C15OH), 3.58 (s, 3 H, H3O), 1.18, 1.19, 1.25, 1.53, 1.53 (5 s, 5 methyls), 2.03 and 2.51 (2 s, 2 acetyl methyls), benzoate group: 7.95 (m, 2 H, H2′), 7.43 (m, 2 H, H3′), 7.56 (m, 1H, H4′); ^{13}C NMR δ 37.1 (C1), 194.3 (C2), 147.8 (C3), 155.2 (C4), 44.1 (C5), 46.4 (C6), 70.8 (C7), 65.1 (C8), 40.8, 40.9, (C9 or C20), 55.4 (C10), 52.3 (C11), 38.2 (C12), 65.9 (C13), 76.8 (C14), 73.5 (C15), 69.4 (C16), 45.7 (C17), 92.7 (C18), 42.1 (C19), 37.6 (C21), 76.2 (C22), 11.2 (C23), 20.6 (C26), 177.1 (C30), 18.9, 20.2, 20.2, 20.9, 20.9 (5 methyl groups), 59.7 (C3-OMe), 21.0 and 21.5 (2 methyl acetates), 170.5 and 170.0 (2, C=O), benzoate group: 129.4 (C1′), 129.6 (C2′), 128.6 (C3′), 133.5 (C4′), 165.9 (C=O); MS (FAB) m/z 751 $[M + H]^+$ (12.6), 691 (4.5), 631 (1.0), 569 (2.3), 509 (3.4), 93 (100).

Spirocaracolitone E (5): mp 231−232.5 °C; $[\alpha]^{25}_D = -6.76$ (*c* 0.004, CH_2Cl_2); IR 2957, 1783, 1738, 1697 cm^{-1}; ^{1}H NMR δ

2.39 (m, 1 H, H1), 3.00 (dd, 1 H, J = 6.0, 18.3 Hz, H1), 2.34 (q, 1 H, J = 7.2 Hz, H4), 1.70 (dd, 1 H, J = 4.4, 12.9 Hz, $H6_{eq}$), 2.39 (m, 1 H, $H6_{ax}$), 5.58 (dt, 1 H, J = 4.4, 10.9 Hz, H7), 2.74 (m, 1 H, H8), 2.74 (m, 1 H, H10), 5.22 (d, 1 H, J = 13, Hz, H11), 2.79 (m, 1 H, H12), 5.21 (brs, 1 H, H15), 5.71 (t, 1 H, J = 2.0 Hz, H16), 2.37 (m, 2 H, H19), 1.85 (d, 1 H, J = 14.8 Hz, H21), 1.93 (m, 1H, H21), 4.90 (m, 1 H, H22), 0.93 (d, 3 H, J = 7.4 Hz, H23), 1.12 (d, 1 H, J = 7.2 Hz, H27), 4.60 and 4.91 (d, 2 H, J = 12.9 Hz, H25), 1.80 (s, 3 H, H27), 1.02, 1.22, 1.33 (3 s, 3 × 3 H, H24, H28, H29), 1.91, 1.94, 1.95, 2.07 (4 s, 4 × 3 H, Me-acetate), 3.58 and 3.59 (2 s, 2 × 3 H, C2-OMe and C3-OMe), tigloyl group: 7.07 (dq, J = 1.4, 7.0, 1 H, H3′), 1.91 (s, 3 H, C2′-Me), 1.81 (d, 3 H, J = 7.0 Hz, H4′); ^{13}C NMR δ 31.4 (C1), 47.6 (C4), 41.1 and 44.3 (C5 and C9), 41.7 (C6), 65.7 (C7), 51.2 and 54.4 (C8 and C10), 82.2 (C11), 44.4 (C12), 54.8 (C-13), 134.5 (C-14), 126.7 (C-15), 69.6 (C-16), 47.8 (C17), 90.5 (C18), 42.7 (C19), 40.6 (C20), 39.7 (C21), 73.3 (C22), 15.0 (C23), 12.7 (C26), 23.1 (C27), 19.3, 20.5 and 22.4 (C24, C28, C29), 177.4 (C30), 20.9, 21.0, 21.1, 21.2 (4 acetyl methyls), 51.5 (C2-OMe and C3-OMe), 174.9, 173.5, 170.1, 169.7, 169.6, 169.5, 168.1 (7 C═O's), tigloyl group: 128.4 (C′2′), 138.5 (C3′), 12.0 (C2′-Me), 14.8 (C4′); MS(CI/ISO) m/z 759 $[MH - 100]^+$ (30.7), 699 (34.2), 639 (25.1), 579 (20.7), 519 (12.8), 101 (100).

Spirocaracolitone F (6): mp 145−151 °C; $[\alpha]^{25}_D = -13.45$ (c 0.006, CH_2Cl_2); IR 2950, 1783, 1737 cm^{-1}; ^{1}H NMR δ 2.43 (m, 1 H, H1), 3.01 (dd, 1 H, J = 6.0, 18.2 Hz, H1), 2.36 (m, 1H, H4), 1.86 (m, 1 H, $H6_{eq}$), 2.56 (dd, 1 H, J = 10.3, 12.8 Hz, $H6_{ax}$), 5.78 (dt, 1 H, J = 4.6, 10.9 Hz, H7), 2.82 (m, 1 H, H8), 4.51 and 4.91 (d, 2 H, J = 12.8 Hz, H25), 2.87 (d, 1 H, J = 11.7 Hz, H10), 5.26 (d, 1 H J = 12.1 Hz, H11), 2.82 (m, 1 H, H12), 5.12 (d, 1 H, J = 1.0 Hz, H15), 5.58 (t, 1 H, J = 2.0, Hz, H16), 2.41 (m, 2 H, H19), 1.89 and 1.95 (m, 1 H, H21), 4.88 (dd, 1 H, J = 1.3, 4.4 Hz, H22), 3.60 and 3.61 (s, 2 × 3 H, C2-OMe and C3-OMe), 0.95 (d, 3 H, J = 7.4 Hz, H23), 1.11 (d, 3 H, J = 7.2 Hz, H26), 1.85 (d, 3 H, J = 1.3 Hz, H27), 1.05, 1.21, 1.38 (s, 3 × 3 H, H24, H28, H29), 1.78, 1.97, 2.00, 2.09 (s, 4 × 3 acetyl methyl), benzoyl group: 8.26 (m, 2 H, H2′), 7.41 (m, 2 H, H3′), 7.53 (m, 1 H, H4′); ^{13}C NMR δ 31.4 (C1), 47.5 (C4), 41.1 and 44.3 (C5 and C9), 41.7 (C6), 66.5 (C7), 51.2 (C8), 54.4 (C10), 82.2 (C11), 44.5 (C12), 54.8 (C13), 134.5 (C14), 127.0 (C15), 69.3 (C16), 47.9 (C17), 90.6 (C18), 42.7 (C19), 40.6 (C20), 39.8 (C21), 73.2 (C22), 15.1 (C23), 12.7 (C26), 23.1 (C27), 19.5, 20.5, and 22.5 (C24, C28, C29), 177.5 (C-30), 20.7, 20.9, 21.1, and 21.4 (4 acetyl methyls), 51.5 and 51.8 (C2-OMe and C3-OMe), 169.3, 169.5, 169.6, 170.0, 173.5, 174.9 (6 C═O's), benzoyl group: 130.0 (C1′), 130.2 (C2′), 128.4 (C3′), 132.7 (C4′), 166.5 (C═O); MS (FAB) m/z 881 $[M + H]^+$ (1.8), 821 (1.3), 761 (4.5), 759 (34.0), 699 (11.5), 639 (7.5), 105 (100).

X-ray Data Collection. Crystals of spirocaracolitones A, B, and E having approximate dimensions o.d. 0.2 by 0.2 by 0.2 mm were mounted on a glass capillary. All the measurements were made on a Rigaku diffractometer with Mo Kα radiation. The three molecules crystallized with solvent molecules: spirocaracolitone A crystallized with one molecule of ethanol and one half molecule of both water and acetone; Spirocaracolitone B, with one half molecule of water; and spirocaracolitone E with one half molecule each of water and ethanol.

Cell constants and an orientation matrix for data collection were obtained from least square refinement using the setting angles of 25 reflections on the range $40 < 2\theta < 50$.

Based on the systematic absences, the space groups were determined to be monoclinic for A and B, and orthorhombic for B. The data were collected at −110 °C using the $\omega-2\theta$ scan technique to a maximum 2θ value of 49.9°. A total of 3832, 3938, and 4726 reflections were collected for A, B, and E, respectively, with 3614, 3938, and 4159 being unique. The standards were measured after every 150 reflections. No crystal decay was noted. The data were corrected for Lorentz and polarization effects.[9] No absorption correction was made. Solution and refinement: The structure was solved by direct methods. All atoms were refined anisotropically except for hydrogen. The hydrogen atoms were calculated. The final cycle of full matrix least squares refinement as based on 2980, 2637, and 3281 reflections ($I < 2.5\sigma I$ and 478, 474, and 577 variable parameters for A, B, and E, respectively. Weights based on counting statistics were used. All of the calculations were performed using the NRCVAX crystallographic software package.[10,11]

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of spiorcaracolitones A−F (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(11) The authors have deposited atomic coordinates for these structures with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.