Antimicrobial Diterpenes of Croton sonderianus, 1. Hardwickic and 3,4-Secotrachylobanoic Acids

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ANTIMICROBIAL DITERPENES OF *CROTON SONDERIANUS*, 1. HARDWICKIC AND 3,4-SECOTRACHYLOBANOIC ACIDS

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ABSTRACT.—The hexane-extracted resin of the roots of *Croton sonderianus* showed antimicrobial activity in standardized bioassays. Fractionation of the resin yielded two acidic diterpenes, (−)-hardwickic acid [**2**] and the new 3,4-secotrachylobanoic acid [**5**] as major bioactive materials.

Croton sonderianus Muell. Arg. (Euphorbiaceae), popularly known as "marmeleiro preto," is a very common shrub of the Northeast region of Brazil. It was once considered a serious weed and was subject to a suggested eradication project. However, its widely dispersed and dense populations underscore how well it has adapted to the drastic climatic conditions of that region. Therefore, it is likely that it plays an important role in the equilibrium of the ecological system. The durable marmeleiro preto is used by the people of the Northeast as fire wood, as weaving material for lobster traps, and as fence posts. Formulations of the plant are also used in popular medicine in the treatment of gastric disturbances. Recently, it has been shown to possess a high content of volatile oil that has potential diesel-like fuel properties. The volatile oil is present in all parts of the plant from the roots through the leaves and is at an average concentration of 1.0% of the dry wt. Gc-ms analysis of the oil has allowed the characterization of 19 monoterpenes and sesquiterpenes (1,2). The seeds are rich in a fixed oil whose fatty acids have been identified (1). From the C_6H_6 extract of the heartwood, a known coumarin, two new cleisthantane diterpenes (4), and a new clerodane diterpene, sonderianin [**1**], were isolated. X-ray crystallography (3) of sonderianin determined its stereochemistry and a structure with a spirolactone ring system.

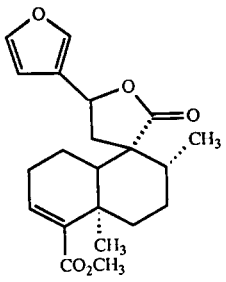
The extracted organic resin from the roots of marmeleiro preto showed significant antimicrobial activity in qualitative biological assays. This observation and the considerations above suggested that a more detailed analysis of the chemical composition was in order. We report herein the characterization of two diterpene acids that are, in part, responsible for the antimicrobial activity of the extracted resin from the roots of *Cr. sonderianus*.

RESULTS AND DISCUSSION

Ground roots of *Cr. sonderianus* afforded a viscous oily extract by cold percolation with hexane. Cold percolation with EtOH of the marc yielded a brown viscous extract after concentration. Both the EtOH and hexane extracts showed biological activity in preliminary qualitative antimicrobial assays using the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the Gram-negative bacteria *Pseudomonas aeruginosa* and *Escherichia coli*, the acid-fast bacterium *Mycobacterium smegmatis*, the yeasts *Candida albicans* and *Saccharomyces cerevisiae*, the filamentous fungus *Aspergillus niger*, the dermatophyte *Trichophyton mentagrophytes*, and the fungal plant pathogens *Polyporus sanguineus* and *Helminthosporium* sp. as test microorganisms.

During concentration of the hexane extract under reduced pressure, there occurred precipitation of a solid material that after filtration and recrystallization from hexane

yielded a white crystalline substance characterized as sonderianin [**1**] by spectroscopic data comparison (ir, ^{1}H and ^{13}C nmr), tlc cochromatography, and mmp determination with an authentic sample. Sonderianin had been previously isolated from the C_6H_6 extract of the heartwood of the same plant (3).



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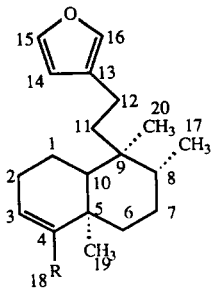


2 R = CO_2H
3 R = CO_2Me
4 R = CH_2OH

The viscous oil remaining after filtration of the sonderianin was separated into acid and neutral fractions, the former exhibiting a somewhat greater biological activity. Extensive chromatography of the acidic fraction led to the isolation of a yellow solid, mp 88–89°, $[M]^+$ (*m/z*) 316 ($C_{20}H_{28}O_3$), which upon CH_2N_2 methylation yielded an ester, $[M]^+$ (*m/z*) 330 ($C_{21}H_{30}O_3$). Spectroscopic analyses of the acid and its methyl ester together with comparison of the data with that reported for (−)-hardwickic acid [**2**], its methyl ester **3**, the alcohol **4**, and its 3,5-dinitrobenzoate derivative indicated it to be hardwickic acid (5,6). Direct comparison of **3** with an authentic sample of the methyl ester of (−)-hardwickic acid proved its identity. (−)-Hardwickic acid and its (+) isomer have been obtained as natural products from families other than Euphorbiaceae, but only (−)-hardwickic acid has been obtained from other *Croton* species.

TABLE 1. Antibacterial Activity[a] of Hardwickic Acid [**2**] and 3,4-Secotrachylobanoic Acid [**5**] and Their Derivatives.

Organism	Compound							
	2	**3**	**4**	**5**	**6**	**7**	**8**	Streptomycin sulfate
Bacillus subtilis								
24 h	+++	+	++	++++	+	++	+++	+++
48 h	+++	+	++	+++	+	++	++	+++
Escherichia coli								
24 h	—	—	—	—	—	—	—	+++
48 h	—	—	—	—	—	—	—	+++
Staphylococcus aureus								
24 h	++	+	++	+++	+	+	—	+++
48 h	++	+	++	+++	+	—	—	+++
Mycobacterium smegmatis								
48 h	+++	1	+++	+++	+	+	++	++++
72 h	+++	1	+++	+++	+	+	—	++++
Pseudomonas aeruginosa								
24 h	—	—	—	—	—	—	—	+++
48 h	—	—	—	—	—	—	—	++

[a]Code: average radius in mm of the zone of inhibition; +, 1–2; ++, 3–6; +++, 7–12; ++++, >12; —, no zone of inhibition observed.

[b]All substances were prepared as solutions in EtOH at concentrations of 1 mg/ml.

No biological properties have been specifically related to hardwickic acid. As can be seen in Tables 1 and 2, (−)-hardwickic acid [**2**] showed a significant qualitative antibacterial activity against the Gram-positive bacteria (*B. subtilis, St. aureus*) and *M. smegmatis*. Its derivatives **3** and **4**, however, were much less potent. Minimum inhibitory concentration (MIC) determination indeed confirmed the high potency of hardwickic acid as a Gram-positive antibacterial (Table 3).

TABLE 2. Antifungal Activity[a] of Hardwickic Acid [**2**] and 3,4-Secotrachlobanoic Acid [**5**] and Their Derivatives.

Organism	Compound						
	2	**3**	**4**	**5**	**7**	**8**	Amphotericin B
Saccharomyces cerevisiae							
47 h	—	—	++	—	—	—	+++
72 h	—	—	++	—	—	—	+++
Candida albicans							
48 h	+	+	++	++	+	+	+++
72 h	++	+	+++	++	+	+	+++
Aspergillus niger							
48 h	—	—	—	—	—	—	+++
72 h	—	—	—	—	—	—	+++
Trichophyton mentagrophytes							
48 h	+++	+	+++	++	—	—	+++
72 h	++	+	++	+	—	—	+++
Polyporus sanguineus							
48 h	—	—	—	++	—	—	++++
72 h	—	—	—	+	—	—	++++
Helminthosporium sp.							
48 h	++	+	++	+	—	—	++++
72 h	++	+	+	—	—	—	++++

[a]Code: average radius in mm of the zone of inhibition; +, 1–2; ++, 3–6; +++, 7–12; ++++, >12; —, no zone of inhibition observed.

[b]All samples were prepared as solutions in EtOH at concentrations of 1 mg/ml.

The second acid, **5**, obtained by Si gel chromatography followed by Florisil chromatography was a clear yellowish oil, $[\alpha]^{23}D$ −44.7° ($c = 5.4$, $CHCl_3$), $[M]^+$ (*m/z*) 302 ($C_{20}H_{30}O_2$), ir (neat) 1700 (C = O), 1650, 1635, and 900 cm^{-1} (C = C). Inspection of the ^{13}C-nmr spectrum revealed the presence of 20 carbons in agreement with the molecular formula suggested by mass spectrometry. Seventeen saturated carbons absorbed in the range of δ 18.6 to 50.9, and 3 sp^2 carbons absorbed at δ 113.8, 147.4, and 181.5. From the single-frequency off-resonance decoupled (SFORD) spectrum analysis and chemical shift theory, one can easily assign δ 181.5 (s) to a carboxylic carbon and δ 113.8 (t) and 147.4 (s) to an isopropylidene moiety. The ^1H-nmr spectrum of the acid showed a broad singlet at δ 11.05 (exchangeable with D_2O, CO_2H), two singlets at δ 0.95 and 1.20 (3H each), two broad singlets at δ 4.70 and 4.90 (one proton each, H-18), and a singlet at δ 1.70 (3H, H-19) characteristic of the isopropylidene group. Ozonolysis of the methyl ester **6** gave the expected methyl ketone **8**, $[M]^+$ (*m/z*) 318 ($C_{20}H_{30}O_3$), showing a carboxymethyl at 1735 cm^{-1} and a carbonyl at 1705 cm^{-1} in its ir spectrum and the corresponding signals at δ 174.0 and 211.5, respectively, in the ^{13}C-nmr spectrum. The ^1H-nmr of ketone **8** contained a methyl absorption at δ 2.10 (CH_3-CO). The ketoester readily reacted with 2,4-dinitrophenlhydrazine to give the phenylhydrazone, which was characterized by ^{13}C- and ^1H-nmr and ms.

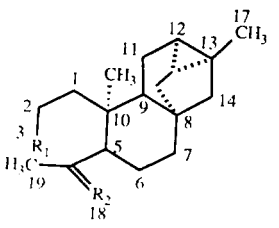
TABLE 3. Minimum Inhibitory Concentrations (MIC) of Hardwickic Acid [**2**] and 3,4-Secotrachylobanoic Acid [**5**] in μg/ml.

Organism	Compound			
	2	**5**	Streptomycin	Amphotericin B
Bacillus subtilis				
24 h	0.78	25.0	3.12	—
48 h	1.56	25.0	3.12	—
Trichophyton mentagrophytes				
24 h	25.0	—	—	0.78
48 h	25.0	—	—	0.78
Saccharomyces cerevisiae				
24 h	—	100.0	—	6.25
48 h	—	100.0	—	12.5

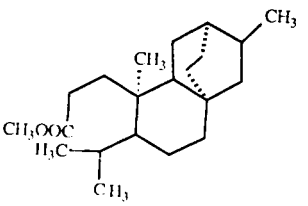
The molecular formula of the natural acid $C_{20}H_{30}O_2$ implies six double bond equivalents. As the carboxy group and the double bond account for two, the acid must be tetracyclic with two angular methyl groups.

CH_2N_2 methylation yielded the expected methyl ester **6**, $[M]^+$ (*m/z*) 316 ($C_{21}H_{32}O_2$), ir 1735 cm^{-1} (C=O), 1635 (C=C), and 3080 cm^{-1} (H-C=). No significant changes were observed in either the ^{1}H- or ^{13}C-nmr spectra other than the presence of the methoxy absorption, δ 3.70 (OCH_3) in the ^{1}H-nmr spectrum, and the expected shift of the carboxyl to δ 174.4 and the additional absorption at δ 51.4 (q in the SFORD, OCH_3) in the ^{13}C-nmr spectrum. However, the product **9** of catalytic hydrogenation of the ester over platinum catalyst showed $[M]^+$ (*m/z*) 320 ($C_{21}H_{36}O_2$) indicative of uptake of 2 molar equivalents of hydrogen. Because the natural acid has only one carbon-carbon double bond, it must have an alternative labile functional group such as a cyclopropane ring to account for the additional hydrogen consumption observed. Indeed, close examination of the ^{1}H-nmr spectrum of the acid **5**, or any of its derivatives other than the hydrogenated product, clearly showed two multiplets around δ 0.65 and 0.85 (1H each) in agreement with a cyclopropane structural feature.

Evaluation of the literature for model compounds yielded trachylobanoic acid [**10**] a pentacyclic diterpene previously obtained from *Trachylobium verrucosum* (Caesalpinaceae) (7) as a good model. Its ^{1}H-nmr spectrum showed three quaternary methyls at δ 0.97, 1.08, and 1.13 and two complex signals centered at δ 0.59 and 0.75 assigned to the cyclopropane hydrogens. As can be seen, except for the methyl at δ 1.08, which is not present in our acid **5**, all the absorptions are in agreement with a 3,4-seco-



5 $R_1 = CO_2H$, $R_2 = CH_2$
6 $R_1 = CO_2Me$, $R_2 = CH_2$
7 $R_1 = CH_2OH$, $R_2 = CH_2$
8 $R_1 = CO_2Me$, $R_2 = O$



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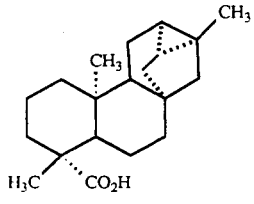
trachylobane type structure for this acid. Trachyloban-19-oic acid was also later obtained from sunflower, *Helianthus annuus* (Compositae) and complete carbon assignments made with the aid of shift reagent and single-frequency selective decoupling (SFSD) techniques (8). The shift reagent $Eu(dpm)_3$ was also used in the assignment of all protons of trachylobanol (9). Comparison of the literature ^{13}C-nmr data of trachyloban-19-oic acid [**10**] (as its methyl ester) with those data observed for acid **5** indeed showed a very good correlation for carbons 7 through 17, which are similar parts of both molecules (Table 4).

TABLE 4. Comparison of the ^{13}C-nmr Assignments of 3,4-Secotrachylobanoic Acid [**5**] with Trachylobanoic Acid Methylester [**10**] and 3,4-Secosonderianol [**11**].

Carbon	Compound		
	11[a] ($CDCl_3$)	**5** (C_6D_6)	**10**[b] ($CDCl_3$)
C-1	34.8	33.7	39.3
C-2	28.6	29.0	18.8
C-3	175.3	181.5	38.2
C-4	141.3	147.4	43.8
C-5	46.6	50.9	52.8
C-6	24.9	26.5	19.7
C-7	29.6	37.9	39.5
C-8	127.0	40.7	40.8
C-9	139.1	44.6	57.0
C-10	41.2	40.3	38.7
C-11	111.6	19.7	21.8
C-12	152.6	20.8	20.6
C-13	120.0	22.5	22.4
C-14	146.9	50.7	50.4
C-15	135.5	24.5	24.3
C-16	119.6	33.5	33.1
C-17	12.9	20.7	20.6
C-18	114.3	113.8	28.7
C-19	22.8	23.6	171.5
C-20	27.9	18.6	10.8
MeO	51.7	—	51.0

[a]Data for this compound are from Craveiro and Silveira (4).
[b]Data for this compound are from Annone *et al.* (9).

3,4-Seco compounds like the triterpenes alnustic (10), dammarenolic acid, and nycthanthic acid (11), the diterpene secobeyerene (12), and the secocleisthantane, 3,4-secosonderianol [**11**] (4) have been reported. Their formation likely results from the photochemical cleavage of the C-3–C-4 bond of 3-keto precursors (11). Comparison of the ^{13}C-nmr absorption of 3,4-secosonderianol [**11**] the latter previously isolated from



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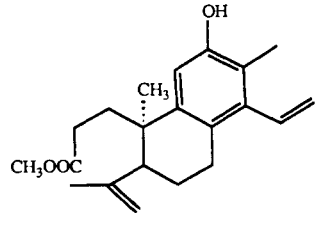

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the wood of marmeleiro preto, with the ^{13}C-nmr absorption of corresponding carbons of the seco portion of the acid **5**, provided compelling support for the structural proposition (Table 4).

3,4-Secotrachylobanoic acid [**5**] also showed significant biological activity. Tables 1 and 2 show its relative antimicrobial activity, and Table 3 shows its MIC values against *B. subtillis* and *Sa. cerevisiae*.

EXPERIMENTAL

PLANT MATERIAL.—The entire roots of *Cr. sonderianus* were used in this study. The whole plant was collected in Sobral, Ceara, Brazil and was identified by Dr. Afranio G. Fernandes. The voucher specimens representing the collection are deposited at the herbarium of the Departmento de Botanica, Universidade Federal do Ceara, Brazil.

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined in either a Fisher-Johns digital melting point analyzer model 355 or a Thomas-Hoover Uni-melt capillary apparatus and were not corrected. Ir spectra were taken as KBr pellets, $CHCl_3$ solutions, or neat films on a Perkin-Elmer 281 B spectrometer. Uv spectra were recorded on a Beckman spectrophotometer Acta III with MeOH solutions. Specific rotations $[\alpha]D$ were obtained on a Perkin-Elmer 141 automatic polarimeter using $CHCl_3$ solutions. Cd curves were recorded on a JASCO J-40 automatic recording spectropolarimeter using MeOH solutions. Ms were obtained on a Finnigan 3200 GC/MS mass spectrometer coupled to an INCOS data system operating in electron impact mode at 70 eV. High resolution mass spectral analyses were conducted in the Department of Chemistry, University of Kansas, Lawrence.

^1H-nmr spectra were recorded on a Varian EM-390 (90 MHz) operating in a CW mode, using $CDCl_3$ solutions and TMS as internal standard. The ^{13}C nmr experiments were performed on a JEOL JNM-FX60 (15.03 MHz) operated in Fourier transform mode using solutions of the same deuterated solvent used for ^1H nmr and TMS as internal standard. For both ^1H and ^{13}C nmr, chemical shifts are expressed in ppm relative to TMS as internal standard (δ units). Proton noise decoupled (PND) spectra were obtained with the FX60 by centering the decoupler frequency 500 Hz below TMS with a noise bandwidth of 1.0 KHz, a pulse angle of 45°, a pulse repetition of 5.0 or 6.0 sec, a sweep width of 4000 Hz, and 8K data points. Single frequency off-resonance decoupled (SFORD) spectra were obtained with the proton decoupler set at 1000 Hz downfield from the signal for TMS. Si gel cc employed MN silica gel 60 (70–270 mesh), 230–400) mesh, or MN Si gel G/UV_{254} for tlc. Tlc analysis was performed by utilizing MN precoated plates, and detection of compounds was achieved by spraying with a prepared solution of EtOH–*p*-anisaldehyde–HOAc (90:5:1) to which 5% by volume of concentrated H_2SO_4 had been added immediately before use. All solvents used for chromatography purposes were A.R. grade or distilled before use.

BIOLOGICAL SCREENING PROCEDURES.—Qualitative and quantitative antimicrobial screening was performed in the Department of Pharmacognosy, University of Mississippi. The assay is based on the general qualitative and quantitative antimicrobial activity bioassay described by Hufford *et al.* (13), as modified by Clark *et al.* (14). The qualitative antimicrobial screening procedure consisted of testing the extract, fractions, and pure compounds against the following microorganisms, obtained from the American Type Culture Collection (ATCC): *B. subtilis* (ATCC 6633), *St. aureus* (ATCC 6538), *E. coli* (ATCC 10536), *Ps. aeruginosa* (ATCC 15442), *M. smegmatis* (ATCC 607), *Ca. albicans* (ATCC 10231), *Sa. cerevisiae* (ATCC 9763), *A. niger* (ATCC 16888), *Tri. mentagrophytes* (ATCC 9972), *Po. sanguineus* (ATCC 16422), and *Helminthosporium* sp. (ATCC 4671).

Plates for the assay were prepared by dispersing 25 ml of sterile agar medium in 100 × 15 mm sterile Petri dishes. Using the quadrant streak method, the sterile agar plates were streaked with a dilution of the test organism (1 ml of broth culture in 9 ml of sterile H_2O) and cultured in eugon agar and eugon broth (for bacteria) or mycophil agar and broth (for fungi). Wells were created in the agar by removal of cylindrical plugs (11 mm diameter) from the solidified agar plates using a sterile cork borer. To the wells were added 100 μl of solution or suspension of an extract, fraction, or pure compound. The extracts and fractions were tested in a concentration of 20 mg/ml; pure compounds were tested at 1 mg/ml. The plates prepared as described were incubated at 37° (for bacteria) or at 30° for fungi and yeasts. The antimicrobial activity was recorded as the width (in mm) of the inhibition zone after 24 h and 48 h of incubation for bacteria, and 48 h and 72 h of incubation for fungi and the *Mycobacterium*. We reported using the following codes: (−) no activity, (+) 1–2 mm, (++) 3–6 mm, (+++) 7–12 mm, and (++++) greater than 13 mm. A standard antibacterial agent, streptomycin sulfate, and a standard antifungal agent, amphotericin B, were included in each assay as positive controls.

A quantitative antimicrobial assay, to determine the MIC of active compounds using the two-fold se-

rial broth dilution assay as previously described (14), was performed for pure compounds that showed significant relative activity in the qualitative screen.

PLANT EXTRACTION.—The roots of *Cr. sonderianus* were air-dried at room temperature and ground. The ground material (1.6 kg dry wt) was then extracted by steam distillation to obtain a light blue volatile oil after H_2O decantation. The residue was air-dried at ambient temperature and extracted by percolation with hexane. The hexane solution upon evaporation under low pressure at 40° yielded 85.0 g of a yellowish resinous extract. The marc obtained after hexane extraction was percolated with 95% EtOH to yield 80.0 g, after EtOH evaporation under vacuum, of a dark brown resinous extract. Both extracts showed antimicrobial activity.

ISOLATION OF SONDERIANIN [**1**].—The concentrated hexane extract was allowed to stand at room temperature for a short time, and a crystalline precipitate began to form. A first crop of sonderianin [**1**] was obtained by filtration of the precipitated material; recrystallization from hexane yielded 2.75 g of **1**: mp 133–135° [lit. (3) mp 134–137°], compared to an authentic sample.

PARTITIONING OF HEXANE EXTRACT.—The resinous filtrate of the hexane extract remaining after recovery of sonderianin (75.0 g) was solubilized in 200 ml of C_6H_6 and stirred for 30 min with 150 ml of 20% NaOH/H_2O solution. The aqueous basic phase was recovered and the treatment repeated twice more with fresh base solution. The remaining C_6H_6 phase was washed with distilled H_2O, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to yield 25.0 g of a clear yellowish oily neutral fraction, which was sealed under dry N_2 and stored at 5°. The combined aqueous alkali solutions were neutralized in the cold with concentrated HCl by dropwise addition. The neutralized solution was extracted three times with 150 ml portions of C_6H_6. The C_6H_6 extracts were combined, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to yield 47.8 g of a clear, brownish resinous acid fraction. Both the neutral and acid fractions showed antimicrobial activity.

INITIAL FRACTIONATION OF THE ACID FRACTION.—The crude acid fraction was coarsely fractionated in the following manner: the acid fraction (30 g) was adsorbed to 30 g of Si gel (70–230 mesh) and placed on top of Si gel (150 g) contained in a 5.5 cm column. Elution with hexane-EtOAc-HOAc (87.5:10.0:2.5) gave twenty 100-ml fractions. Four additional 150-ml fractions were obtained by elution with hexane-EtOAc-MeOH (4:3:3). Comparison of the collected fractions by tlc and ^{1}H nmr allowed combination into 8 pooled fractions, F1/1 through F1/8.

ISOLATION OF HARDWICKIC ACID [**2**].—A portion (5.7 g) of the third pooled fraction, F1/3, was adsorbed onto 10 g of Si gel and chromatographed over 100 g of Si gel for tlc (column diameter 5.5 cm). The column was eluted with hexane-$CHCl_3$ (1:1), and 35 20-ml fractions were collected after which the column was washed with MeOH/$CHCl_3$. The fractions were combined after tlc and ^{1}H-nmr comparison to give seven similar pooled fractions, F2/1 through F2/7. The fifth fraction, F2/5 (670 mg), was adsorbed onto 6.7 g of Florisil and chromatographed over 50 g of Florisil for tlc (column diameter 2.5 cm). Elution with $CHCl_3$ gave 34 20-ml fractions. From fractions 16 to 20, 152 mg of homogeneous (−)-hardwickic acid [**2**] was obtained in solid form, mp 88–89° [lit. (5) 106–107° or 99–100°], which upon attempted recrystallization could never be recovered crystalline; $[\alpha]^{23}D$ −85.5° [lit. −102.3°], ir ν max (film) 3500–3100 (sh), 2650–2500 (sh), 1685, 1500, 1260, 1030, 880 cm^{-1}; uv λ max MeOH (log ϵ) 213 nm (3.83); ms *m/z* (rel. int.) $[M]^+$ 316 (5.3), $[M-Me]^+$ 301 (1.1), $[M-Me-H_2O]^+$ 283 (5.1), 221 (26.0), 203 (21.9), 175 (19.6), 125 (90.5), 95 (100), 81 (70); ^{1}H nmr ($CDCl_3$), δ 12.1 (1H, br s, exchangeable with D_2O, acid H), 7.37 (1H, m, H-15), 7.23 (1H, br s, H-16), 6.90 (1H, t, J = 3.0 Hz, H-3), 6.25 (1H, m, H-14), 1.27 (3H, s, H-19), 0.85 (3H, d, J = 6.0 Hz, H-17), 0.80 (3H, s, H-20); ^{13}C nmr ($CDCl_3$) δ 173.1 (s, C-18), 143.5 (d, C-16), 142.8 (s, C-4), 139.4 (d, C-15), 138.1 (d, C-3), 126.5 (s, C-13), 11.8 (d, C-14), 47.6 (d, C-10), 39.7 (s, C-9), 39.5 (t, C-11), 38.3 (s, C-5), 37.0 (t, C-6), 36.6 (d, C-8), 28.0 (t, C-2), 27.1 (t, C-7), 20.9 (q, C-19), 18.6 (t, C-1, C-12), 18.2 (q, C-20), 16.2 (q, C-17). Comparison with authentic sample made as methyl ester (see below).

The same Florisil column was washed with 250 ml of 10% MeOH in $CHCl_3$ followed by 250 ml of $CHCl_3$ and used to recycle the combined fractions 21 to 34. Elution with 1% MeOH in $CHCl_3$ yielded 391 mg more of (−)-hardwickic acid [**2**].

HARDWICKIC ACID METHYL ESTER [**3**].—Hardwickic acid [**2**], 120 mg, was diluted in 15 ml of Et_2O and methylated with excess CH_2N_2. Filtration of the reaction residue over a small column of Si gel yielded 110 mg of **3** as a yellowish oil: $[\alpha]^{23}D$ −104° (c = 1.1, $CHCl_3$); uv λ max MeOH (log ϵ) 214 nm (3.38); CD (c = 14.0, MeOH) $\Delta\epsilon_{239}$ −4.43; ir ν max (neat) 1710, 1630, 1500, 1250, 1230, 875 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 330 (2.5), $[M-15]^+$ 315 (0.4), $[M-OMe]^+$ 299 (2.3), $[M-Me-MeOH]^+$ 283 (8.6), $[M-95]^+$ 235 (24.6), 203 (37.9), 139 (100), 96 (55.0), 95 (54.0), 81 (50); ^{1}H nmr ($CDCl_3$) δ 7.37 (1H, m, H-15), 7.21 (1H, br s, H-16), 6.50 (1H, t, J = 3.5 Hz, H-3), 6.26 (1H, m, H-14), 3.65

(3H, s, OCH_3), 1.25 (3H, s, H-19), 0.85 (3H, d, J = 6.0 Hz, H-17), 0.78 (3H, s, H-20); ^{13}C nmr ($CDCl_3$) δ 167.8 (s, C-18), 142.7 (d, C-16), 142.7 (s, C-4), 138.5 (d, C-15), 136.7 (d, C-3), 125.7 (s, C-13), 111.0 (d, C-14), 51.1 (q, OCH_3), 46.8 (d, C-10), 39.0 (s, C-9), 38.9 (t, C-11), 37.7 (s, C-5), 36.5 (d, C-8), 36.1 (t, C-6), 27.3 (t, C-2), 27.5 (t, C-7), 20.5 (q, C-19), 18.3 (q, C-20), 18.3 (t, C-1), 17.7 (t, C-12), 16.0 (q, C-17). Comparison made with authentic methyl hardwickiate.

HARDWICKIOL [**4**].—(−)-Hardwickic acid [**2**] (120 mg), was dissolved in Et_2O and reduced with 50 mg of $LiAlH_4$ to yield 95 mg of hardwickiol [**4**] as a colorless oil: $[\alpha]^{23}D$ −41.8° (c = 7.0, $CHCl_3$); ir ν max (neat) 3310, 1500, 1450, 1380, 1030, 875 cm^{-1}; ms m/z (rel. int.) $[M]^+$ 302 (2.6), $[M-Me]^+$ 287 (1.2), $[M-CH_2OH]^+$ 271 (4.8), $[M-95]^+$ 207 (3.9), $[M-H_2O-95]^+$ 189 (32.4), 95 (100); ^{1}H nmr ($CDCl_3$) δ 7.40 (1H, m, H-15), 7.25 (1H, br s, H-16), 6.30 (1H, br s, H-14), 5.60 (1H, t, J = 3.0 Hz, H-3), 4.15 (2H, br s, H-18), 2.2 (1H, br s, O*H* exchangeable with D_2O), 1.10 (3H, s, H-19), 0.84 (3H, d, J = 7.0 Hz, H-17), 0.79 (3H, s, H-20). ^{13}C nmr ($CDCl_3$) δ 148.3 (s, C-4), 142.7 (d, C-16), 138.5 (d, C-15), 125.8 (s, C-13), 121.8 (d, C-3), 111.0 (d, C-14), 62.8 (t, C-18), 46.5 (d, C-10), 38.8 (s, C-9), 38.8 (t, C-11), 37.9 (s, C-5), 36.5 (d, C-8), 36.5 (t, C-6), 27.4 (t, C-2), 26.6 (t, C-7), 21.4 (q, C-19), 18.3 (q, C-20), 18.3 (t, C-1, C-12), 16.0 (q, C-17).

3,5-Dinitrobenzoate, mp 115–118°, cochromatography and mmp comparison with authentic sample.

ISOLATION OF 3,4-SECOTRACHYLOBANOIC ACID [**5**].—Fraction F2/3 (1.4 g) was absorbed onto 10 g of Si gel for flash chromatography (230–400 mesh), then chromatographed on a column of 100 g of the same silica previously deactivated with 20 g of H_2O. Elution with 1% MeOH in $CHCl_3$ yielded thirteen 20-ml fractions. Following tlc and ^{1}H-nmr comparison, similar fractions were combined to form four pooled fractions, F3/1 through F3/4.

Fraction F3/3 (612 mg) was rechromatographed on the recovered Florisil column as above to yield 210 mg of hardwickic acid [**2**] and 325 mg of **5** as a colorless oil: $[\alpha]^{23}D$ −44.7° (c = 5.4, $CHCl_3$); ir ν max (neat) 3300–3000 (br sh), 2550 (sh), 1705, 1635, 1445, 900 cm^{-1}; ms m/z (rel. int.) $[M]^+$ 302 (4.7), $[M-Me]^+$ 287 (0.9), $[M-Me-CH_2=CH_2]^+$ 259 (1.2), $[M-HCO_2-CH_2=CH_2]^+$ 229 (6.0), 201 (7.3), 175 (21.3), 174 (21.3), 173 (16.7), 119 (38.4), 105 (100), 91 (64.5); ^{1}H nmr δ ($CDCl_3$) 11.8 (1H, br s, CO_2H), 4.90 (1H, m, H-18*Z*), 4.70 (1H, br s, H-18*E*), 1.70 (3H, br s, H-19), 1.20 (3H, s, H-17), 0.95 (3H, s, H-20), 0.85 (1H, m, H-12), 0.60 (1H, m, H-15); ^{13}C nmr see Table 4; hrms m/z calcd for $C_{20}H_{30}O_2$ $[M]^+$ 302.2246, found $[M]^+$ 302.2237.

3,4-SECOTRACHYLOBANOIC METHYL ESTER [**6**].—The acid **5** (220 mg) was dissolved in Et_2O and treated with an excess of CH_2N_2. Usual workup yielded 210 mg of the ester as a colorless oil: $[\alpha]^{23}D$ −38.9° (c = 10.6, $CHCl_3$); ir ν max (neat) 1740, 1445, 1375, 1120, 890 cm^{-1}; ms m/z (rel. int.) $[M]^+$ 316 (2.5), $[M-Me]^+$ 301 (0.5), $[M-OMe]^+$ 285 (0.3), $[M-Me-CH_2=CH_2]^+$ 273 (0.9), $[M-HCO_2CH_3-CH_2=CH_2]^+$ 229 (4.4), 201 (8.9), 175 (19.2), 119 (38.9), 105 (100), 91 (59.1); ^{1}H nmr δ ($CDCl_3$) 4.93 (1H, m, H-18*Z*), 4.73 (1H, br s, H-18*E*), 3.70 (3H, s, OCH_3), 1.76 (3H, br s, H-19), 1.17 (3H, s, H-17), 1.00 (3H, s, H-20), 0.85 (1H, m, H-12), 0.65 (1H, m, H-15); ^{13}C nmr δ ($CDCl_3$) 174.4 (s, C-3), 147.4 (s, C-4), 113.4 (t, C-18), 51.4 (q, OMe), 50.9 (d, C-5), 50.5 (t, C-14), 44.4 (d, C-9), 40.5 (s, C-8), 40.1 (s, C-10), 37.8 (t, C-7), 33.6 (t, C-1), 33.2 (t, C-16), 28.6 (t, C-2), 26.2 (t, C-6), 24.2 (d, C-15), 23.4 (s, C-13), 22.3 (q, C-19), 20.4 (q, C-17), 20.4 (d, C-12), 19.5 (t, C-11), 18.4 (q, C-20).

4-OXO-3,4-SECONORTRACHYLOBAN-3-OIC METHYL ESTER [**8**].—3,4-Secotrachylobanoic methyl ester [**6**] (120 mg) was dissolved in 10 ml of CH_2Cl_2. The solution was cooled in a dry ice-Me_2CO bath and treated with ozone until a blue color was developed by the solvent. To the reaction mixture was added 0.5 ml of Me_2S, and the mixture was stirred for 1.0 h at room temperature. The solvent was evaporated in a stream of N_2. To the residue, 0.5 ml of Me_2S and 10 ml of MeOH were added, and the solution was refluxed overnight. After evaporation of the MeOH, the residue was filtered through a column of Florisil (2 g) to yield 85 mg of **8** as a yellowish oil: ir ν max (neat) 1735, 1710, 1440, 1195, 1175, 795, 720 cm^{-1}; ms m/z (rel. int.) $[M]^+$ 318 (7.2), $[M-Me]^+$ 303 (0.5), $[M-OMe]^+$ 287 (2.9), $[M-MeOH]^+$ 286 (4.6), 274 (5.8), 270 (4.3), 259 (4.0), 231 (10.9), 159 (77.1), 105 (79.6) 97 (100), 87 (99.8); ^{1}H nmr (90 MHz, $CDCl_3$) δ 3.65 (3H, s, OCH_3), 2.10 (3H, s, H-19), 1.15 (3H, s, H-17), 1.04 (3H, s, H-20), 0.83 (1H, m, H-12), 0.63 (1H, m, H-15); ^{13}C nmr ($CDCl_3$) δ 211.5 (s, C-4), 174.1 (s, C-3), 56.7 (d, C-5), 51.4 (q, OMe), 50.5 (t, C-14), 44.2 (d, C-9), 40.3 (s, C-8), 39.7 (s, C-10), 36.8 (t, C-7), 34.2 (t, C-16), 33.1 (t, C-1), 31.2 (q, C-19), 29.7 (t, C-6), 28.6 (t, C-2), 24.1 (d, C-15), 23.3 (s, C-13), 20.3 (q, C-17), 20.3 (d, C-12), 19.2 (t, C-11), 18.1 (q, C-20).

TETRAHYDRO-3,4-SECOTRACYLOBANOIC ACID METHYL ESTER [**9**].—The acid **5** (80 mg) was dissolved in 15 ml of MeOH; PtO_2 (15 mg) was added and the mixture was stirred for 2 h under an atmosphere of H_2 at atmospheric pressure. The reaction mixture was filtered through a small column of silica (2 g) for

removal of catalyst to yield 75 mg of tetrahydro-3,4-secotrachylobanoic acid methyl ester [**9**] as a colorless oil: $[\alpha]^{23}D$ −11.3° (*c* = 15.0, $CHCl_3$); ir ν max (neat) 1740, 1470, 1445, 1180, 850, 760 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 320 (3.0), $[M-H_2]^+$ 318 (3.0), $[M-H_2-OMe]^+$ 287 (1.8), $[M-MeCH]^+$ 262 (22.6), 231 (35), 203 (19.7), 175 (39.6), 106 (100), 93 (60); ^1H nmr ($CDCl_3$), δ 3.67 (3H, s, OMe), 1.15 (3H, s, H-20), 0.90 (6H, d, *J* = 8.0 Hz, H-18, -19), 0.80 (3H, d, *J* = 7.0 Hz, H-17).

3,4-SECOTRACHYLOBANOL [**7**].—3,4-Secotrachylobanoic acid [**5**] (80 mg) was dissolved in Et_2O and reduced with 50 mg of $LiAlH_4$. The resultant alcohol **7** (65 m)g was obtained as a colorless oil: $[\alpha]^{23}D$ −47.0° (*c* = 12.0, $CHCl_3$); ir ν max (neat) 3300, 1630, 1445, 1380, 890, 760 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 288 (3.8), $[M-Me]^+$ 273 (0.6), 229 (3.9), 201 (6.8), 119 (39.6), 105 (100), 91 (58.0); ^1H nmr ($CDCl_3$) δ 4.85 (1H, m, H-18*Z*), 4.67 (1H, br s, H-18*E*), 3.50 (2H, t, *J* = 7.0 Hz, H-3), 1.73 (3H, br s, H-19), 1.15 (3H, s, H-17), 0.90 (3H, s, H-20), 0.80 (1H, m, H-12), 0.63 (1H, m, H-15); ^{13}C nmr ($CDCl_3$) δ 147.9 (s, C-4), 112.9 (t, C-18), 63.6 (t, C-3), 50.7 (d, C-5), 50.4 (t, C-14), 44.2 (d, C-9), 40.5 (s, C-8), 40.2 (s, C-10), 37.8 (t, C-7), 34.6 (t, C-1), 33.1 (t, C-16), 26.5 (t, C-2), 26.2 (t, C-6), 24.2 (d, C-15), 23.2 (s, C-13), 22.2 (q, C-19), 20.5 (q, C-17), 20.5 (d, C-12), 19.4 (t, C-11), 18.9 (q, C-20).

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LITERATURE CITED

1. A.A. Craveiro, E.R. Silveira, F.J.A. Matos, and J.W. Alencar, *Rev. Latinoam. Quim.*, **9**, 95 (1978).
2. E.R. Silveira, "Contribuição ao Conhecimento Químico de Plantas Nativas do Nordeste: *Croton sonderianus*," MSc. Thesis presented to Universidade Federal do Ceara, Fortaleza, 1979.
3. A.A. Craveiro, E.R. Silveira, R. Braz-Filho, and Y.P. Mascarenhas, *Phytochemistry*, **20**, 852 (1981).
4. A.A. Craveiro and E.R. Silveira, *Phytochemistry*, **21**, 2571 (1982).
5. R. Misra, R.C. Pandey, and S. Dev, *Tetrahedron*, **35**, 979, 985, 2301 (1979).
6. P.R. Jefferies, J.R. Know, and B. Scaf, *Aust. J. Chem.*, **26**, 2199 (1973).
7. G. Hugel, L. Tods, J.M. Mellor, D.W. Theobald, and G. Ourisson, *Bull. Soc. Chim. Fr.*, 2888 (1965).
8. J. St. Pyrek, *Tetrahedron*, **26**, 5029 (1970).
9. A. Annone, R. Mondelli, and J. St. Pyrek, *Org. Magn. Reson.*, **12**, 429 (1979).
10. T. Suga, T. Aoki, Y. Kawada, and E. Ohta, *Phytochemistry*, **23**, 1297 (1984).
11. D. Arigoni, D.H.R. Barton, R. Bernasconi, C. Djerassi, J.S. Mills, and R.E. Wolff, *J. Chem. Soc.*, 1900 (1960).
12. E.L. Ghisalberthi, P.R. Jefferies, and M.A. Sefton, *Phytochemistry*, **17**, 1961 (1978).
13. C.D. Hufford, M.J. Funderburk, J.M. Morgan, and L.W. Robertson, *J. Pharm. Sci.*, **64**, 789 (1975).
14. A.M. Clark, F.S. El-Feraly, and W.-S. Li, *J. Pharm. Sci.*, **70**, 951 (1981).

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