New Bioactive Coumarins from *Kielmeyera albopunctata*

Elita Scio,[†,‡] Antônia Ribeiro,[†] Tânia M. A. Alves,[†] Alvaro J. Romanha,[§] Young G. Shin,[⊥] Geoffrey A. Cordell,[⊥] and Carlos L. Zani*[,†]

Laboratório de Química de Produtos Naturais, CPqRR-FIOCRUZ, Avenida Augusto de Lima, 1715, 30190-002, Belo Horizonte, MG, Brazil, Departamento de Bioquímica, ICB–Universidade Federal de Juiz de Fora, 36036-030, Juiz de Fora, MG, Brazil, Laboratório de Parasitologia Celular e Molecular, CPqRR-FIOCRUZ, Avenida Augusto de Lima, 1715, 30190-002, Belo Horizonte, MG, Brazil, and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612-7231

Received December 31, 2002

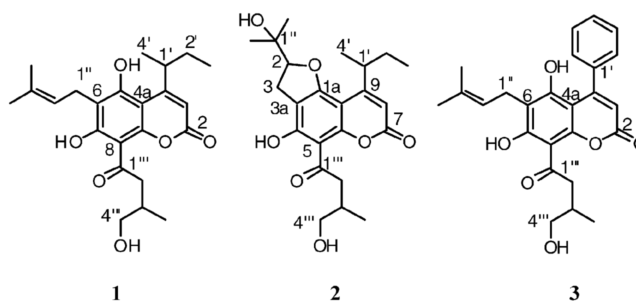
The CH_2Cl_2 extract of the stem bark of *Kielmeyera albopunctata* was subjected to a bioassay-linked LC-MS dereplication procedure using the KB cell line to afford the new coumarins 4-(1-methylpropyl)-5,7-dihydroxy-8-(4-hydroxy-3-methylbutyryl)-6-(3-methylbut-2-enyl)chromen-2-one (**1**), 9-(1-methylpropyl)-4-hydroxy-5-(4-hydroxy-3-methylbutyryl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuro[2,3-*f*]chromen-7-one (**2**), and 5,7-dihydroxy-8-(4-hydroxy-3-methylbutyryl)-6-(3-methylbut-2-enyl)-4-phenylchromen-2-one (**3**). Coumarins **1** and **3** showed moderate cytotoxicity, while **2** was inactive at 20 μg/mL. Compound **1** was active in vitro against the trypomastigote form of the parasite *Trypanosoma cruzi*, killing 80% of the parasites after 24 h contact at 4 °C when added at 125 μg/mL to infected murine blood.

Plants from the genus *Kielmeyera* (Clusiaceae) are largely spread in the central Brazilian plateau (cerrado) and are known to be a rich source of xanthones.[1] Several species are known as "pau-santo" and have shown antifungal properties against *Cladosporium cucumerinum* and *Candida albicans*.[2,3] Extracts of *K. coriacea* also offer protection against infection by cercariae of *Schistosoma mansoni* when applied to the skin of experimental animals.[4]

In a continuing program to discover novel bioactive natural products, it was found that the CH_2Cl_2 extract of the stem bark of *K. albopunctata* Saddi showed moderate activity against several human cancer cell lines. Since no previous chemical or biological investigations could be found for this species, it was decided to conduct the bioassay-guided fractionation of this extract using the KB cell line, following a bioassay-linked LC-MS dereplication protocol.[5] The pure compounds were evaluated for their activity against the KB (human oral epidermoid cancer), Col2 (human colon cancer), LNCaP (human prostate cancer), and Lu1 (human lung cancer) cell lines. As the extract was also active against the trypomastigote form of the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas' disease (American trypanosomiasis), the trypanocidal activity of **1**–**3** was also studied.

Results and Discussion

The stem bark of *K. albopunctata* was dried, triturated, and extracted sequentially with hexane and CH_2Cl_2. The hexane extract yielded crystals on standing, which were identified as friedelin by analysis of its ^1H and ^{13}C NMR spectra and by direct comparison with a standard sample. Both the crude hexane extract and friedelin were inactive at 20 μg/mL when tested against the KB, Col2, LNCaP, and Lu1 cell lines using established protocols[6] (data not shown). On the other hand, the CH_2Cl_2 extract showed significant toxicity against some of these cell lines, with ED_{50} values between 11 and 20 μg/mL (Table 1).



Using the KB cell line, this extract was investigated employing the bioassay-linked LC-MS dereplication protocol[5] to simultaneously detect and gain structural information on the compounds present in the active fraction (Figure 1 in the Supporting Information). Compound **2** was isolated from an inactive fraction eluting at 18.15 min.

Compound **1** was obtained as a yellow amorphous solid. Its HRFAB mass spectrum showed $[M + H]^+$ at m/z 403.2132, consistent with the molecular formula $C_{23}H_{30}O_6$. The UV spectrum of **1** in MeOH showed absorption maxima at 219, 295, and 331 nm. The IR spectrum of **1** showed bands indicating the presence of hydroxyl groups (ν_{max} 3406 cm^{-1}), an α,β-unsaturated lactone (ν_{max} 1701 cm^{-1}), and a chelated acyl group (ν_{max} 1604 cm^{-1}).[7–9] Together with the ^1H and ^{13}C NMR spectra, these data indicated the presence of a coumarin skeleton with all positions substituted, except C-3. The HMBC contour map showed correlations between the H-3 singlet (δ 5.87) and the lactone carbonyl carbon (C-2, δ 161.2), the methine carbon (C-1′, δ 37.3), and the quaternary carbon (C-4a, δ 104.9). The COSY contour map showed a correlation between H-1′ (δ 4.50, 1H, m), H-4′ (δ 1.17, 3H, d, $J = 6.8$), and H-2′a (δ 1.73, 3H, m). These COSY correlations and the other HMBC correlations of H-1′ allowed the location of a 1-methylpropyl substituent at C-4 of the coumarin skeleton.

HMBC experiments also showed correlations of the methylene proton H_2-1″ (δ 3.38, 2H, d, $J = 5.8$ Hz) with C-6 (δ 112.9) and the C-5 and C-7 oxygenated carbons of the heterocycle (δ 167.8). Correlations between H-2″

* Corresponding author. Fax: +55 31 3295 3115. E-mail: zani@cpqrr.fiocruz.br.
† Laboratório de Química de Produtos Naturais.
‡ Universidade Federal de Juiz de Fora.
§ Laboratório de Parasitologia Celular e Molecular.
⊥ University of Illinois at Chicago.

10.1021/np020597r CCC: $25.00 © 2003 American Chemical Society and American Society of Pharmacognosy
Published on Web 05/01/2003

Table 1. Activity of Extract and Pure Compounds from *Kielmeyera albopunctata* against Human Cancer Cell Lines[a] and *Trypanosoma cruzi*[b]

	cell line[a]				*T. cruzi*[b]	
	Lu1	Col2	KB	LNCaP	500 μg/mL	125 μg/mL
CH_2Cl_2 extract	14.7	11.1	20	12.2	99	
1	19.4	13.1	15.8	14.0	100	80
2	>20	>20	>20	>20		
3	19.8	17.4	17.7	15.9	98	

[a] Lu1 (lung), Col2 (colon), KB (oral epidermoid), and LNCaP (prostate). Results expressed as ED_{50} (effective dose to inhibit cell proliferation to 50%). Highest dose used was 20 μg/mL. Ellipticine was used as positive control with ED_{50} 0.02, 0.3, 0.04, and 0.8 μg/mL for Lu1, Col2, KB, and LNCaP, respectively. [b] Trypomastigote form of *T. cruzi* Y strain in infected murine blood. Results expressed as percent of lysis of parasites after 24 h contact at 4 °C. Gentian violet at 7.5 μg/mL was used as positive control, causing 50% lysis.

(δ 5.20, 1H, m), H-4″ (δ 1.73, 3H, s), and H-5″ (δ 1.60, 3H, d, J = 0.8 Hz) were also evident. Together, these data allowed placement of a prenyl substituent at the C-6 position.

The methylene protons H-2‴b (δ 2.69, 1H, dd, J = 14.0 and 8.4 Hz) and H-2‴a (δ 3.52, 1H, dd, J = 14.0 and 5.1 Hz) showed HMBC correlations with the C-1‴ carbonyl (δ 204.7), the C-3‴ methine (δ 34.3), the C-4‴ methylene (δ 67.8), and the C-5‴ (δ 17.2) methyl groups. Together with the COSY analysis, these data supported the presence of a 4-hydroxy-3-methylbutyryl moiety in the molecule. The hydroxyl groups attached to the aromatic carbons were located at C-5 and C-7 on the basis of the UV data, ^{13}C NMR shifts, and HMBC correlations. The placement of this substituent at C-8 was based on the fact that no bathocromic shift of the UV bands was observed after the addition of alkali, a behavior that is similar to that reported for a number of 8-acyl-5,7-dioxygenated coumarins.[10-14] The complete assignment of the ^1H and ^{13}C NMR signals (see Experimental Section) was based on detailed interpretation of HMQC, HMBC, $^1H-^1H$ COSY, and TOCSY experiments and is compatible with the proposed structure. Acetylation of **1** afforded the tri-*O*-acetate derivative **1a**, with methyl signals at δ_H 2.03, 2.23, and 2.38 typical for one aliphatic and two aromatic acetate moieties. On the basis of this analysis, compound **1** was identified as 4-(1-methylpropyl)-5,7-dihydroxy-8-(4-hydroxy-3-methylbutyryl)-6-(3-methylbut-2-enyl)chromen-2-one.

Compound **2**, isolated as an amorphous yellow powder, generated (1:1) doubled signals in the ^{13}C NMR spectrum, suggesting that **2** was a mixture of isomers. Attempts to separate this mixture using normal- and reversed-phase HPLC columns were not successful. Hereafter, one of the doubled signals observed for this compound is shown within square brackets.

The molecular formula of **2** was established as $C_{23}H_{30}O_7$ by HRFABMS ($[M + H]^+$ 419.2070). The IR spectrum exhibited main absorptions at 3406 (OH), 1710 (α,β-unsaturated lactone), 1600 (chelated acyl group), and 1393 cm^{-1} (geminal dimethyl). Comparison of the ^1H NMR data of **1** and **2** revealed that the latter also had a 1-methylpropyl group. The presence of a 4-hydroxy-3-methylbutyryl group was evidenced by the HMBC data, showing a correlation between the methylene protons H-2‴a and H-2‴b (δ 3.55 [3.71] and 2.64 [2.55], respectively) of the acyl chain with a carbonyl carbon (C-1‴, δ 205.6 [206.1]) and a methine carbon (C-3‴, δ 32.8 [33.2]). This group was located at C-5 on the basis of the UV spectrum of **2** (λ_{max} 217 and 296 nm) and literature data for similar structures.[10-14]

The ^1H NMR spectrum showed a signal at δ 14.21 [14.26], attributed to a chelated hydroxyl group at C-4 (δ 163.2 [162.9]). The absence of any other phenolic hydroxyl group and the presence of an oxygenated carbon at C-2a (δ 162.2 [162.3]) suggested the formation of a ring involving the C-2a and C-3a positions at the *f* face of the coumarin skeleton. The alternative ring formation at the *g* face was discarded, taking into account the presence of a chelated OH group (δ_H 14.21 [14.26]). In the HMBC contour plot, the methyl protons at δ 1.43 (3H, s, H-3″) and 1.29 (3H, s, H-2″) correlated with a tertiary alcohol function at C-1″ (δ 71.6 [71.8]) and with a tertiary carbon at C-2 (δ 92.9 [92.9]). The signal of H-3 (δ 3.23 [3.12]) showed cross-peaks with C-2 and C-1″ (δ 92. 9 [92.9], 71.6 [71.8], respectively) and with C-2a (δ 162.2 [162.3]) and C-3a (δ 110.7 [110.7]) from the benzenoid ring. These data confirmed the presence of a dihydrofuran ring substituted with a 1-hydroxy-1-methylethyl group and fused in the *f* face of the coumarin. The structure of 9-(1-methylpropyl)-4-hydroxy-5-(4-hydroxy-3-methylbutyryl)-2-(1-hydroxy-1-methylethyl)-2,3-dihydrofuro[2,3-*f*]chromen-7-one is proposed for the compound mixture **2**. It is plausible to propose that **2** is derived from **1** via epoxidation of the prenyl side chain followed by cyclization via attack of the C-5 OH to form the dihydrofuran ring with a 1-hydroxy-1-methylethyl side chain.

Compound **3** showed a $[M + Na]^+$ adduct in the HRFABMS with *m*/*z* 445.1633, compatible with the molecular formula $C_{25}H_{26}O_6$. The IR spectrum showed absorptions at ν_{max} 3472 (OH), 1710 (α,β-unsaturated lactone), and 1592 (aromatic) cm^{-1}. The ^1H NMR spectrum of **3** exhibited signals at δ 5.98 (1H, s, H-3) and 7.47 (5H, m, H-2′ to H-6′), indicating the presence of a phenyl group. Comparison of the NMR data of **1** and **3** revealed that **3** also had 4-hydroxy-3-methylbutyryl and 3-methyl-2-butenyl substituents. Indeed, COSY correlations were observed between H-2″ and H-1″, H-4″, and H-5″. The HMBC contour map showed correlations between H-1″ and C-5, C-6, C-7, C-2″, and C-3″. Other HMBC correlations supported the presence of a prenyl group at C-6. The methylene protons of H-2‴ (δ 3.55 and 3.03) showed correlations in the HMBC contour map with C-1‴, C-3‴, C-4‴, and C-5‴. Other HMBC correlations confirmed the nature of the acyl substituent. The ^1H NMR revealed two low-field singlets at δ 14.70 and 7.46 due to phenolic hydroxyls, the former being chelated. It is known that in the 4-phenyl series this difference in chemical shift is observed only in 8-acyl-6-prenylcoumarins and not in 6-acyl-8-prenylcoumarins.[13] As was the case for **1**, the 8-acyl-5,7-dihydroxycoumarin chromophore in **3** was evidenced by the lack of bathochromic shift in the UV spectrum after alkali addition.[11,12,15,16] On the basis of this analysis, compound **3** was identified as 5,7-dihydroxy-8-(4-hydroxy-3-methylbutyryl)-6-(3-methylbut-2-enyl)-5-phenylchromen-2-one.

The absolute stereochemistry of the methyl groups in compounds **1**−**3** could not be deduced from the available data. Compounds **1**−**3** showed small $[\alpha]_D$ values, as described for a number of other closely related coumarins isolated from the genera *Mammea*,[10,12] *Mesua*,[7,17] and *Calophylum*.[18] Compounds **1**−**3** differ from those isolated from Crombie et al.[11-14] in the hydroxylation of the 3-methylbutyryl side chain to form the 4-hydroxy-3-methylbutyryl group, the position of the acyl group, and the methylation of the propyl side chain to form the 1-methylpropyl substituent.

Compounds **1**−**3** were evaluated for their cytotoxicity, and the results are summarized in Table 1. Activity of the pure compounds was similar to that of the crude CH_2Cl_2 extract from which they were isolated. This might be explained by a synergistic effect between these compounds and other components of the extract or by the presence of a potent minor component that was missed during the isolation procedure.

Chagas' disease (American trypanosomiasis) is caused by the protozoan parasite *T. cruzi* and affects approximately 18 million people in Central and South America. When subjected to the infected murine blood assay, the CH_2Cl_2 extract and compounds **1** and **3** showed significant trypanocidal activity when tested at 500 μg/mL, killing 99, 100, and 98% of the parasites, respectively (Table 1). However, at lower concentrations only **1** was able to retain some activity, causing the lysis of 80% of the parasites at 125 μg/mL.

Experimental Section

General Experimental Procedures. Melting points were determined using a Fisher-Johns MP apparatus and are uncorrected. The UV spectra were obtained on a Beckman DU-7 spectrophotometer. NMR spectra were recorded with a Bruker Avance DPX-300 spectrometer at 300 MHz, with TMS as the internal standard. IR spectra were recorded on a Mattson-Galaxy series FTIR 3000, and optical rotations were measured on a Perkin-Elmer 241 polarimeter. LREIMS (70 eV) were recorded on a Shimadzu GCMS-QP 5050 gas chromatography mass spectrometer, and HRFABMS were recorded on a Finnigan MAT 90 spectrometer. HPLC-electrospray/MS chromatograms and spectra for dereplication were measured on a Hewlett-Packard 5989B single quadrupole mass spectrometer coupled with a 59987A electrospray interface and a Hitachi HPLC L-7100. Mixtures of acetonitrile−H_2O were used as eluent. TLC was run on precoated silica gel plates, eluting with DCM−MeOH mixtures in different proportions and vanillin−H_2SO_4 as visualization reagent. Analytical and semipreparative HPLC were run on a Shimadzu chromatograph equipped with a LC10AD pump and a UV detector at 210 and 254 nm. MeOH was used as eluent. Analytical (7.6 × 200 mm) and semipreparative (20 × 250 mm) columns (Shodex-Asahipack GS 310 kit) were used. Centrifugal planar chromatography was carried out on a Harrison Research Chromatotron and eluted with DCM−MeOH in different proportions. Medium-pressure liquid chromatography was run using a Büchi column (36 × 230 mm) packed with silica gel 60 and eluted with mixtures of hexane−CH_2Cl_2−MeOH.

Plant Material. The stem bark of *K. albopunctata* was collected from Reserva Florestal de Linhares, Espírito Santo, Brazil, in 1997. The plant material was identified by Prof. Júlio Lombardi of the Federal University of Minas Gerais. A voucher specimen was deposited at BHCB (Herbarium of the Biological Sciences Institute of Federal University of Minas Gerais, Brazil) under the code BHCB 0260.

Extraction and Separation. The dried and powdered stem bark (250 g) of *K. albopunctata* was extracted with hexane (1 L) followed by dichloromethane (1 L) and evaporated to dryness under vacuum. The hexane fraction (4 g) was subjected to medium-pressure liquid chromatography. Gradient elution using hexane−CH_2Cl_2−MeOH yielded eight fractions. Fraction 5 (185 mg), eluted with CH_2Cl_2−MeOH (85:15), afforded the triterpene friedelin (20.2 mg) after crystallization from hexane−CH_2Cl_2 (1:1). The CH_2Cl_2 extract (200 mg) was submitted to silica radial centrifuge chromatography under N_2 atmosphere using mixtures of CH_2Cl_2−MeOH (100:0, 98:2, 90:10, 50:50, and 0:100) to afford 11 fractions. Fraction 3 (60 mg) and fraction 4 (20 mg), eluted with CH_2Cl_2−MeOH (98:2 and 90:10, respectively), were subjected to further purification using semipreparative HPLC. Fraction 3 yielded coumarins **1** (7 mg) and **3** (2 mg), and fraction 4 furnished coumarin **2** (9 mg).

Friedelin: white crystals from hexane−CH_2Cl_2; showed physical and chemical data identical with published data.[19]

Compound 1: yellow amorphous solid; mp 135−136 °C; $[\alpha]_D$ +3.8° (MeOH, *c* 0.105); UV (MeOH) λ_{max} (log ϵ) 219 (4.37), 295 (4.08), 331 (4.24); IR (KBr) ν_{max} cm^{-1}: 3406, 1701, 1604, 1393; ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 5.87 (1H, s, H-3), 5.20 (1H, m, H-2″), 4.50 (1H, m, H-1′), 3.56 (1H, dd, *J* = 10.6, 5.8 Hz, H-4‴a), 3.52 (1H, dd, *J* = 14.0, 5.1 Hz, H-2‴a), 3.40 (1H, dd, *J* = 10.6, 7.1 Hz, H-4‴b), 3.38 (2H, d, *J* = 5.8 Hz, H-1″), 2.69 (1H, dd, *J* = 14.0, 8.4 Hz, H-2‴b), 2.35 (1H, m, H-3‴), 1.73 (3H, s, H-4″), 1.73 (1H, m, H-2′a), 1.60 (3H, d, *J* = 0.8 Hz, H-5″), 1.37 (1H, m, H-2′b), 1.17 (3H, d, *J* = 6.8 Hz, H-4′), 0.98 (3H, d, *J* = 6.2 Hz, H-5‴), 0.90 (3H, t, *J* = 7.4 Hz, H-3′); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 204.7 (C, C-1‴), 168,0 (C, C-4), 167.8 (C, C-5 and C-7), 161.2 (C, C-2), 158.5 (C, C-8a), 131.5 (C, C-3″), 124.1 (CH, C-2″), 112.9 (C, C-6), 105.0 (CH, C-3), 104.9 (C, C-4a), 102.7 (C, C-8), 67.9 (CH_2, C-4‴), 49.1 (CH_2, C-2‴), 37.3 (CH, C-1′), 34.3 (CH, C-3‴), 30.6 (CH_2, C-2′), 25.9 (CH_3, C-5″), 22.3 (CH_2, C-1″), 20.3 (CH_3, C-4′), 18.1 (CH_3, C-4″), 17.2 (CH_3, C-5‴), 12.3 (CH_3, C-3′); HRFABMS *m/z* 403.21325 [M + H]$^+$ (calcd for $C_{23}H_{31}O_6$, 403.21206); FABMS *m/z* [M + H]$^+$ 403.3 (65), 329.2 (100), 245.1 (22), 176.1 (38), 154.1 (47), 136.1 (41), 107.0 (30), 77.1 (45), 55.7 (29).

Compound 2: yellow amorphous powder (MeOH); $[\alpha]_D$ 0° (MeOH, *c* 0.27); UV (MeOH) λ_{max} (log ϵ) 217 (3.81), 296 (3.90); IR (KBr) ν_{max} cm^{-1} 3406, 1710, 1600, 1393; ^{1}H NMR ($CDCl_3$, 300 MHz) δ14.21 [14.26] (1H, s, OH-4); 6.07 [6.01] (1H, s, H-8), 4.87 (1H, dd, *J* = 9.5, 8.6 Hz, H-2), 3.70 [3.79] (1H, m, H-4‴b), 3.60 (1H, m, H-1′), 3.55 [3.71] (1H, m, H-2‴a), 3.30 [3.39] (1H, m, H-4‴a), 3.23 [3.12] (2H, m, H-3), 2.64 [2.55] (1H, m, H-2‴b), 2.40 (1H, m, H-3‴), 1.84 [1.75] (2H, m, H-2′), 1.43 (3H, s, H-3″), 1.29 (3H, s, H-2″), 1.26 [1.19] (3H, d, *J* = 6.8 Hz, H-4′), 0.98 [1.04] (3H, d, *J* = 7.4 [7.2] Hz, H-3′), 0.97 [0.91] (3H, d, *J* = 6.4 [6.6] Hz, H-5‴); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.6 [206.1] (C, C-1‴), 163.8 [164.2] (C, C-9), 163.2 [162.9] (C, C-4), 162.2 [162.3] (C, C-2a), 160.9 [161.0] (C, C-7), 156.9 [157.0] (C, C-5a), 110.7 [110.7] (C, C-3a), 106.2 [106.3] (CH, C-8), 105.0 [105.3] (C, C-5), 99.4 [99.5] (C, C-9a), 92.9 [92.9] (CH, C-2), 71.6 [71.8] (C, C-1″), 68.1 [68.1] (CH_2, C-4‴), 48.7 [48.7] (CH_2, C-2‴), 37.3 [37.4] (CH, C-1′), 32.8 [33.2] (CH, C-3‴), 28.7 [29.5] (CH_2, C-2′), 26.4 [26.5] (CH_2, C-3), 26.0 [26.3] (CH_3, C-3″), 24.6 [24.7] (CH_3, C-2″), 19.2 [19.5] (CH_3, C-4′), 16.2 [16.5] (CH_3, C-5‴), 11.8 [11.9] (CH_3, C-3′); HRFABMS *m/z* 419.20695 [M + H]$^+$ (calcd for $C_{23}H_{31}O_7$, 419.20698); FABMS *m/z* [M + H]$^+$ 419.2 (20), 400.2 (10), 345.1 (8), 307.1 (31), 289.1 (23), 254.1 (100), 136.1 (78), 124.1 (20), 107.0 (40), 77.0 (38).

Compound 3: pale yellow powder (MeOH); mp 141−142 °C; $[\alpha]_D$ +2.1° (acetone, *c* 0.14); UV (MeOH) λ_{max} (log ϵ) 230 (3.93), 286 (3.86), 334 (3.87); IR ν_{max} cm^{-1} 3472, 1710, 1592; ^{1}H NMR [$(CD_3)_2CO$, 300 MHz] δ 14.70 (1H, s, OH-7), 7.47 (4H, m, H-2′, H-3′, H-5′ and H-6′), 7.46 (1H, s, OH-5), 5.98 (1H, s, H-3), 5.07 (1H, tbr, *J* = 6.6, Hz, H-2″), 3.55 (1H, dd, *J* = 15.5, 5.5 Hz, H-2‴a), 3.55 (2H, dd, *J* = 9.8, 6.0 Hz, H-4‴), 3.37 (2H, dbr, *J* = 6.6 Hz, H-1″), 3.30 (1H, s, OH-4‴), 3.03 (1H, dd, *J* = 15.5, 7.8 Hz, H-2‴b), 2.41 (1H, m, H-3‴), 1.71 (3H, sbr, H-5″), 1.63 (3H, sbr, H-4″), 1.05 (3H, d, *J* = 6.8 Hz, H-5‴); ^{13}C NMR [$(CD_3)_2CO$, 75 MHz] δ 205.9 (C, C-1‴), 167.5 (C, C-7), 159.3 (C, C-8 a), 158.9 (C, C-5), 157.1 (C, C-2), 156.8 (C, C-4), 140.2 (CH, C-1′), 133.1 (C, C-3″), 129.5 (CH, C-2′and C-6′), 129.3 (CH, C-4′), 129.0 (CH, C-3′ and C-5′), 122.1 (C-2″), 112.9 (CH, C-3), 112.7 (C, C-6), 105.1 (C, C-8), 102.5 (C, C-4 a), 67.5 (CH_2, C-4‴), 49.3 (CH_2, C-2‴), 34.0 (CH, C-3‴), 25.8 (CH_3, C-4″), 21.9 (CH_2, C-1″), 18.0 (CH_3, C-5″), 17.2 (CH_3, C-5‴); HRFABMS *m/z* 445.16329 [M + Na]$^+$ (calcd for $C_{25}H_{26}O_6Na$, 445.16271); FABMS *m/z* [M + H]$^+$ 423.2 (38), 349.2 (41), 307.1 (27), 154.1 (100), 136.1 (64), 89.0 (24), 51.3 (12).

Acetylation of 1. Compound **1** (1 mg) was stirred at room temperature with acetic anhydride (0.5 mL) and pyridine (0.5 mL) for 24 h. Removal of excess solvent and reagent yielded peracetate **1a** as a pale yellow powder: $[\alpha]_D$ +2.1° (MeOH, *c* 0.1); ^{1}H NMR (MeOH 300 MHz) δ 6.40 (1H, s, H-3), 4.87 (1H, overlapped with OH signal from solvent, H-2″), 4.00 (1H, m, H-1′), 3.17 (2H, brs, H-4‴), 3.15 (1H, d, *J* = 5.8 Hz, H-1″a), 3.09 (1H, d, *J* = 5.8 Hz, H-1″b), 2.89 (1H, d, *J* = 7.4 Hz, H-2‴a), 2.83 (1H, d, *J* = 7.4 Hz, H-2‴b), 2.47 (1H, q, *J* = 6.7 Hz, H-3‴),

2.38 (3H, s, CH_3CO-7), 2.23 (3H, s, CH_3CO-5), 2.03 (3H, s, CH_3-CO-4″), 1.73 (3H, s, H-3″), 1.67 (3H, s, H-5″), 1.55 (2H, m, H-2′), 1.28 (3H, d, $J = 6.7$ Hz, H-4′), 1.06 (3H, d, $J = 6.8$ Hz, H-5‴), 0.97 (3H, t, $J = 7.3$ Hz, H-3′); HRFABMS *m*/*z* 529.2419 $[M + H]^+$ (calcd for $C_{29}H_{37}O_9$, 529.24376).

Dereplication. The dereplication method was carried out using HPLC-electrospray/MS according to a previously published protocol.[5]

Cytotoxic Activity. Established protocols[6] were used. The panel of tumor cell lines used included the Lu1 (human lung cancer), Col2 (human colon cancer), LNCaP (human prostate cancer), and KB (human oral epidermoid carcinoma) cell lines.

Trypanocidal Activity. The assays with *T. cruzi* were carried out using the blood of Swiss albino mice collected in the parasitaemia peak (seventh day) after infection with the Y strain of *T. cruzi*. The infected blood was diluted with normal murine blood to the concentration of 2×10^6 trypomastigotes/mL. Stock solutions (20 mg/mL) of the CH_2Cl_2 extract and compounds **1**−**3** were prepared in dimethyl sulfoxide (DMSO). A 5.0 mL portion of each solution was added to 195 mL of infected blood, providing a final concentration of 500 mg/mL. Samples of 100 mL were transferred in duplicate to the wells of a microtiter plate (96 wells). To reproduce the blood bank conditions, plates were incubated at 4 °C for 24 h. The experiments were repeated three times. The parasite concentration was evaluated according to the procedure described by Brener.[20] DMSO at 2.5% v/v and gentian violet at its IC_{50} concentration (7.5 mg/mL) were used as negative and positive controls, respectively. The trypanocidal activity was expressed as a percent reduction of the parasite number (lysis) comparing the wells with the samples to the wells with DMSO alone. DMSO at 2.5% causes no harm to the parasites, erythrocytes, or leukocytes. The samples that caused about 100% reduction in parasite number were tested at lower concentrations.

Acknowledgment. We thank R. O. Alves for technical assistance with *T. cruzi*; Dr. J. M. Pezzuto and the staff of the Bioassay Research Facility, PCRPS-UIC, for the cytotoxic assays; Ms. Mary Lou Quinn for access to the NAPRALERT database; CAPES, CNPq, and Fulbright for fellowships; and PRONEX and FIOCRUZ for financial support.

Supporting Information Available: Dereplication data and tables with complete 1D and 2D NMR data for compouds **1**−**3** are available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Peres, V.; Nagem, T. G. *Phytochemistry* **1997**, *44*, 191−214.
(2) Homans, A. L.; Fuchs, A. J. *J. Chromatogr.* **1970** *51*, 327−329.
(3) Rahalison, L.; Hamburger, M.; Hostettmann, K.; Monod, M.; Frenk, E. *Phytochem. Anal.* **1991**, *2*, 199−203
(4) Lopes, J. L. C.; Lopes, J. N. C.; Gilbert, B.; Bonini, S. E. *Phytochemistry* **1977**, *16*, 1101.
(5) Cordell, G. A.; Beecher, C. W. W.; Kinghorn, A. D.; Pezzuto, J. M.; Constant, H. L.; Fang, L.; Seo, E.-K.; Long, L.; Cui, B.-L.; Barritos, K. S. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; 1997; Vol 19, pp 749−791.
(6) Likhitwitayawuid, K.; Angerhofer, C. K.; Cordell, G. A.; Pezzuto, J. M. *J. Nat. Prod.* **1993**, *56*, 30−38.
(7) Govindachari, T. R.; Pai, B. R.; Subramanian, P. S.; Ramdas Rao, U.; Muthukumaraswamy. *Tetrahedron* **1967**, *23*, 4161−4165.
(8) Bordoloi, M.; Mohan, S.; Barua, N. C.; Dutta, S. C.; Mathur, R. K.; Ghosh, A. C. *Phytochemistry* **1997**, *44*, 939−942.
(9) Cao, S.-G.; Sim, K.-Y.; Pereira, J.; Goh, S.-H. *Phytochemistry* **1998**, *47*, 1051−1055.
(10) Joshi, B. S.; Kamat, V. N.; Govindachari, T. R.; Ganguly, A. K. *Tetrahedron* **1969**, *25*, 1453−1458.
(11) Crombie, L.; Jones, R. C. F.; Palmer, C.J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 345−351.
(12) Crombie, L.; Games, D. E.; McCormick, A. *J. Chem. Soc.* **1967**, *23*, 2545−2552.
(13) Crombie, L.; Jones, R. C. F.; Palmer, C. J. *J. Chem. Soc., Perkin Trans. 1* **1987**, 317−331.
(14) Crombie, L.; Games, D. E.; Haskins, N. J.; Reed, G. F. *J. Chem. Soc., Perkin Trans. 1* **1972**, *18*, 2248−2254.
(15) Thebtaranonth, C.; Imraporn, S.; Padungkul, N. *Phytochemistry* **1981**, *20*, 2305−2306.
(16) Cruz, F. G.; Moreira, L. M.; David, J. M.; Guedes, M. L. S.; Chávez, J. P. *Phytochemistry* **1998**, *47*, 1363−1366.
(17) Morel, C.; Guilet, D.; Oger, J. M.; Séraphin, D.; Sévenet, T.; Wiart, C.; Hadi, A. H. A.; Richomme, P.; Bruneton, J. *Phytochemistry* **1999**, *50*, 1243−1247.
(18) Guilet, D.; Hélesbeux, J. J.; Séraphin, D.; Sévenet, T.; Richomme, P.; Bruneton, J. *J. Nat. Prod.* **2001**, *64*, 563−568.
(19) Queiroga, C. L.; Silva, G. F.; Dias, P. C.; Possenti, A.; Carvalho, J. E. *J. Ethnopharmacol.* **2000**, *72*, 465−468.
(20) Brener, Z. *Rev. Inst. Med. Trop.* **1962**, *4*, 389−396.

NP020597R