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A NEW ANTIMALARIAL QUASSINOID FROM *SIMABA GUIANENSIS*

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ABSTRACT.—Two antimalarial quassinoids, gutolactone [**1**] and simalikalactone D [**2**], have been characterized by bioactivity-directed fractionation from the bark of *Simaba guianensis* collected near Manaus, Brazil. Compound **2** was previously isolated from *Simaba multiflora* and *Quassia africana* and shown to be an active antimalarial in vitro. This is the first occurrence of **1**. The structure of the novel quassinoid was established by spectral methods including 2D nmr spectroscopy.

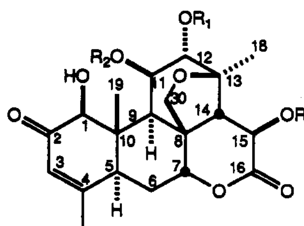
In an ongoing search for antimalarial agents from naturally occurring sources, the bark of *Simaba guianensis* Aubl. (Simaroubaceae) collected near Manaus, Brazil, was selected for investigation. This plant, a small tree that occurs in the flooded areas of the Amazon basin, is locally known as "Cajurana." Its red fruits that ripen during the time of flooding are highly appreciated by fishes and its bark is very bitter and is used by native populations against fevers (1). Cajurana was collected together with ten other plants, all of which have histories of use for treatment of fevers (2). This paper describes the bioassay-guided isolation and structural determination of the novel gutolactone [**1**] and the known simalikalactone D [**2**].

RESULTS AND DISCUSSION

The EtOH extract of the bark of *S. guianensis*, when assayed in vitro for antimalarial activity, demonstrated a potency of one to three orders of magnitude higher than the other plants utilized against "fevers" (Table 1).

Since a preliminary phytochemical examination indicated the presence of alkaloids in this extract, and knowing the canthinones have been isolated from the genus *Simaba* (3–6), the initial fractionation was directed toward the isolation of these compounds (7) following a modified version of the procedure reported for the first isolation of canthin-6-one [**4**] (8) (Scheme 1).

Fractionation of the EtOH extract of the trunk bark of *S. guianensis* into strong bases,

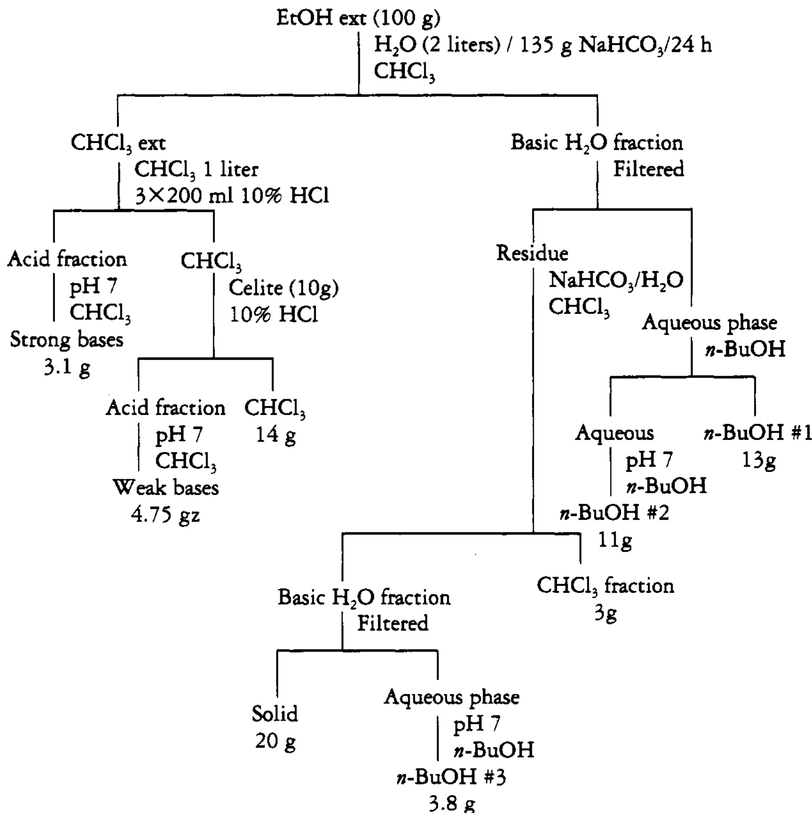


1 R=Me$_2$C=CHCO, R$_1$=R$_2$=H
2 R=MeCH$_2$CH(Me)CO, R$_1$=R$_2$=H
3 R=MeCH$_2$CH(Me)CO, R$_1$=R$_2$=Ac

TABLE 1. In Vitro Antimalarial Activity of Plant Extracts.

Plant extract or standard	*Plasmodium falciparum* strain	
	Indochina W-2 IC_{50} (ng/ml)	Sierra Leone D-6 IC_{50} (ng/ml)
Simaba guianensis Aubl.	930.1	469.3
Geissospermum sericeum (Sagot.) Benth. et Hook	5,632.6	2,156.2
Stryphnodendron guyanense (Aubl.) Benth.	38,903.0	pa[a]
Aspidosperma oblongum A. DC.	4,742.5	847.4
Doliocarpus dentatum (Aubl.) Standl.	11,641.0	15,273.0
Jacaranda copaia (Aubl.) D. Don	20,827.0	32,981.0
Pithecelobium racemosum Ducke	185,040.0	i[b]
Pothomorphe peltata (L.) Miq.	9,078.8	pa
Tynanthus elegans (Cham.) Miers	807,030.0	i
Carapa guianensis Aubl	32,037.0	11,640.0
Peltogyne paniculata Benth.	123,869.0	120,.520.0
Chloroquine	52.4	2.8
Mefloquine	2.0	6.1
Artemisinin	1.6	1.3
Quinine	56.2	8.4

[a]Partially active.
[b]Inactive.



SCHEME 1. Alkaloid fractionation.

weak bases, and neutral fractions furnished eight fractions which were submitted for in vitro antimalarial bioassay. The results are shown in Table 2. This indicated that the antimalarial activity was concentrated in the "weak bases" fraction (IC_{50} 23.4–12.0 ng/ml), which was subjected to further fractionation in order to isolate the substance(s) responsible for the antimalarial activity. The other fractions were set aside.

To focus quickly on the active constituent(s) of the weak bases fraction, it was chromatographed on preparative tlc. Thirteen zones were identified by examination under uv light and were separated, worked up, and sent for antimalarial assay. The results reported in Table 3 indicated that the activity was concentrated in fraction F7 (IC_{50} 5.5–4.3 ng/ml).

Chromatography of this mixture using circular centrifugal chromatography yielded 56 mg of gutolactone [**1**] and 60 mg of simalikalactone D [**2**].

The physical and spectral data of **2** matched those of simalikalactone D in all respects (9). Comparison with an authentic sample of simalikalactone D confirmed the proposed structure of **2**. Simalikalactone D was previously isolated from *Simaba multiflora* A. Juss. in 1985 by Arisawa *et al.* (5) and from *Quassia africana* Baill. by Tresca *et al.* (9), and shown to be active against malaria in vitro by O'Neill *et al.* (10).

Compound **1** was slightly more polar than simalikalactone D. Eims revealed a molecular ion of *m/z* 476, and hrms measurements confirmed an exact mass of 476.2045, showing it has a molecular formula $C_{25}H_{32}O_8$. Since this mol wt is two units lower than that for **2** and its ^{1}H-nmr spectrum was almost superimposable (Table 4), **1** was initially regarded as a "dehydrosimalikalactone D." A more careful examination of the ^{1}H-nmr spectrum of this compound revealed that it contained neither angelic nor tiglic acid, the unsaturated forms of the butane-2 carboxylic acid of the simalikalactone D side chain, but rather senecioic acid. NOESY experiments showed a strong nOe between the two methyl singlets at δ 2.19 and δ 1.95 of **1**; in contrast, there was no nOe between the methyl triplet at δ 0.95 and the methyl doublet at δ 1.2 of **2**. The relative stereochemistry of carbons 1, 5, 7, 8, 9, 10, 11, 12, 13, 14, and 15 in both structures was confirmed with the help of NOESY spectra. The NOESY spectra showed strong interaction between protons at δ 4.16 (H-1), δ 2.94 (H-5), and δ 2.32 (H-9), indicating that all have the same α orientation; the methyl protons at δ 1.42 (H-18) interact with protons at δ 3.78 (H-12) and at δ 2.47 (H-14); and there is also interaction between protons at δ 2.32 (H-9) and δ 6.15 (H-15), showing that H-15 is also α-oriented. Table 5 shows

TABLE 2. In Vitro Antimalarial Activity of Fractions from Scheme 1.

Fraction	*Plasmodium falciparum* strain	
	Indochina W-2 IC_{50} (ng/ml)	Sierra Leone D-6 IC_{50} (ng/ml)
Solid residue	pa[a]	pa
n-BuOH 1	5923.5	6,052.1
n-BuOH 2	7,079.0	6,003.0
n-BuOH 3	14,343.9	22,443.0
$CHCl_3$	7,079.0	20,247.2
Weak bases	23.4	12.0
Strong bases	588.2	570.0
Chloroquine	67.2	2.9
Mefloquine	1.5	8.7
Artemisinin	1.1	2.1
Quinine	69.6	12.0

[a]Partially active.

TABLE 3. In Vitro Antimalarial Activity of the Tlc Fractionation of the Weak Bases.

Fraction	*Plasmodium falciparum* strain	
	Indochina W-2 IC_{50} (ng/ml)	Sierra Leone D-6 IC_{50} (ng/ml)
F1	pa[a]	i[b]
F2	pa	pa
F3	pa	pa
F4	pa	pa
F5	pa	pa
F6	102.2	100.0
F7	5.5	4.3
F8	99.3	35.0
F9	pa	990.0
F10	513.3	451.0
F11	1,475.9	pa
F12	1,576.1	848.2
F13	2,970.7	pa
Choroquine	54.2	2.7
Mefloquine	1.5	8.7
Artemisinin	1.3	2.3
Quinine	58.5	10.4

[a]Partially active.
[b]Inactive.

assignments of the ^{13}C-nmr data for compound **1** and the not previously published assignments for simalikalactone D and its diacetate **3**. The new compound was proposed to have structure **1** and was named gutolactone.

TABLE 4. ^{1}H-nmr Spectral Data (300 MHz, $CDCl_3$) for Compounds **2** and **1**.

Proton	Compounds	
	2	**1**
H-1	4.16 (1H, s)	4.07 (1H, s)
H-3	6.10 (1H, brm)	6.00 (1H, s)
H-5	2.94 (1H, brd, J=12 Hz)	2.86 (1H, d, J=12 Hz)
H-6	2.31 (1H, m)[a]	2.31 (1H, m)[a]
	1.81 (1H, m)[a]	1.72 (1H, t, J=12 Hz)[a]
H-7	4.67 (1H, brdd, J=2.7, 2.7 Hz)	4.61 (1H, s)
H-9	2.31 (1H, m)	2.25 (1H, m)
H-11	3.77 (1H, brs)	3.69 (1H, s)
H-12	4.63 (1H, brs)	4.56 (1H, bs)
H-14	2.37 (1H, m)	2.38 (1H, d, J=9.6 Hz)
H-15	6.16 (1H, brd, J=12 Hz)	6.05 (1H, bs)
H-17	4.62 (1H, m)[a]	3.44 (1H, d, J=7.8 Hz)
	3.53 (1H, m)[a]	3.44 (1H, d, J=7.8 Hz)[a]
4-Me	1.96 (3H, s)	1.82 (3H, s)
10-Me	1.18 (3H, s)	1.09 (3H, s)
13-Me	1.43 (3H, s)	1.33 (3H, s)
H-2′	2.47 (1H, m)	5.67 (1H, s)
H-3′	1.74 (1H, m)[a]	—
	1.50 (1H, m)[a]	—
H-4′	0.95 (3H, t, J=7.5 Hz)	2.10 (3H, s)
H-5′	1.22 (3H, d, J=7.0 Hz)	1.84 (3H, s)

[a]Chemical shift determined by HETCOR and COSY.

TABLE 5. ^{13}C-nmr Spectral Data for Compounds **1**, **2** and **3**.[a]

Carbon	Compounds		
	2	**3**	**1**
C-1	81.7 (1)	82.2 (1)	81.6 (1)
C-2	197.2 (0)	190.5 (0)	197.4 (0)
C-3	124.2 (1)	126.2 (1)	124.2 (1)
C-4	163.5 (0)	160.0 (0)	163.6 (0)
C-5	43.5 (1)	43.3 (1)	43.5 (1)
C-6	28.3 (2)	27.7 (2)	28.3 (2)
C-7	83.1 (1)	82.7 (1)	83.2 (1)
C-8	47.7 (0)	46.0 (0)	47.7 (0)
C-9	42.5 (1)	42.3 (1)	42.4 (1)
C-10	45.9 (0)	45.5 (0)	45.8 (0)
C-11	79.4 (1)	78.9 (1)	79.3 (1)
C-12	74.4 (1)	70.9 (1)	74.3 (1)
C-13	80.4 (0)	79.9 (0)	80.4 (0)
C-14	52.4 (1)	53.0 (1)	52.3 (1)
C-15	67.3 (1)	66.3 (1)	66.8 (1)
C-16	167.7 (0)	166.8 (0)	168.2 (0)
C-17	71.7 (2)	72.3 (2)	71.7 (2)
4-Me	22.6 (3)	22.4 (3)	22.6 (3)
10-Me	22.9 (3)	22.6 (3)	22.8 (3)
13-Me	16.6 (3)	12.1 (3)	11.4 (3)
C-1′	175.4 (0)	175.0 (0)	165.0 (0)
C-2′	41.1 (1)	41.1 (1)	114.7 (1)
C-3′	26.5 (2)	26.4 (2)	160.1 (0)
C-4′	11.6 (3)	11.7 (3)	20.6 (3)
C-5′	11.4 (3)	16.6 (3)	27.6 (3)
*Me*CO	—	20.9 (3)	—
	—	20.8 (3)	—
Me*C*O	—	170.2 (0)	—
	—	169.7 (0)	—

[a]Recorded at 75 MHz in $CDCl_3$. Number of attached protons were determined by APT and LH-HETCOR.

Compounds **1** and **2** were submitted for antimalarial bioassay. The results are shown in Table 6. Both compounds presented in vitro antimalarial activity similar to or better than that of known antimalarials used as standards (IC_{50} 3.9–4.1 ng/ml for gutolactone [**1**] and 1.6–1.5 ng/ml for simalikalactone D [**2**]). Most notably, the activity was the same for the two different strains of *Plasmodium falciparum* tested: the W-2 Indochina, a chloroquine-resistant strain, and the D-6 Sierra Leone, a mefloquine-resistant strain.

TABLE 6. In Vitro Antimalarial Activity of the Constituents of Fraction F7.

Compound	*Plasmodium falciparum* strain	
	Indochina W-2 IC_{50} (ng/ml)	Sierra Leone D-6 IC_{50} (ng/ml)
Simalikalactone D [**2**]	1.6	1.5
Gutolactone [**1**]	4.0	4.1
Chloroquine	63.2	2.9
Mefloquine	1.5	8.7
Artemisinin	1.1	2.1
Quinine	68.6	12.0

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns digital melting point analyzer model 355 and were not corrected. Ir spectra were taken as KBr pellets or $CHCl_3$ solutions on a Perkin-Elmer 281B spectrometer. Uv spectra were taken on a Perkin -Elmer Lambda 3B UV/VIS spectrophotometer in MeOH or EtOH solutions. Specific rotations $[\alpha]D$ were measured on a Perkin-Elmer 141 automatic polarimeter using MeOH solutions. Low resolution eims as obtained on a Finningan 3200 GC/MS mass spectrometer coupled to an INCOS or Teknivent Vector One data system operating at 70 eV and on a Thermospray LC-MS Vestec model 201 coupled to a Waters 600-MS HPLC system. Hrms was obtained in the Department of Chemistry, University of Kansas, at Lawrence. ^{1}H-nmr and ^{13}C-nmr spectra and 1D and 2D experiments were performed on a Varian VXR-300 instrument (300 MHz for ^{1}H and 75 MHz for ^{13}C). For both ^{1}H- and ^{13}C-nmr spectra the chemical shifts are expressed in ppm relative to TMS. For ^{1}H-nmr spectra the descriptions are s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, brs=broad singlet, dd=double doublet, and dt=double triplet. The coupling constants are reported in Hz. For the ^{13}C-nmr spectra the numbers 0, 1, 2, and 3 refer to the number of bonded protons determined by the attached proton test (APT) or distortionless enhancement by polarization transfer (DEPT) experiments. NOESY, COSY, and HETCOR experiments were used for proton and carbon assignments. LR-HETCOR was optimized for ^3J. Cc employed Si gel 60 (70–270 or 230–240 mesh ASTM) or silica HF_{254}. Both analytical and preparative tlc analyses were performed by utilizing MN precoated plates with detection of compounds by examination of the plates under uv light or by spraying with Dragendorff's spray reagent (11). All solvents used for chromatographic purposes were AR grade.

ANTIMALARIAL IN VITRO BIOASSAY.—The antimalarial bioassay was done at the Malaria Research Laboratory of the Division of Experimental Therapeutics of the Walter Reed Army Institute of Research in Washington, DC. Basically it follows the method developed by Desjardins *et al.* (12) and modifications introduced by Milhous *et al.* (13) and is designed to assess the intrinsic activity of candidate antimalarial drugs relative to known controls such as chloroquine mefloquine, artemisinin, and quinine.

SAMPLE PREPARATION.—Crude extracts, fractions, and pure compounds were solubilized in DMSO and diluted 400-fold (to rule out a DMSO effect) in culture medium with plasma for a starting concentration of 125,000 ng/ml. Test materials were subsequently diluted five-fold using the Cetus Pro/Pette System utilizing a range of concentrations from 0.8 ng/ml to 125,000 ng/ml. Inhibitory concentrations of 50% are reported in ng/ml.

ORGANISMS.—Two *P. falciparum* clones were utilized in this assay: the W-2 Indochine *P. falciparum* clone is resistant to chloroquine, pyrimethamine, and sulfadoxine but susceptible to mefloquine, and the D-6 African (Sierra Leone) *P. falciparum* clone is susceptible to chloroquine, pyrimethamine, and sulfadoxine but resistant to mefloquine. The cultures were obtained from the Malaria Laboratory, Division of Experimental Therapeutics, WRAIR/WRAMC, Washington, D.C.

ANTIMALARIAL ACTIVITY.—Uptake of [G-^{3}H]hypoxanthine was used as an index of growth of the parasites. The final isotope solution consisted of 20 μCi of [G-^{3}H]hypoxanthine per ml of culture medium. After the incubation period of 24, 40, 48 or 60 h, the inhibition of uptake of the radiolabeled nucleic acid precursor of both test and controls was automatically measured. A graphic computer output was generated for each test sample which calculates the IC_{50} in ng/ml.

ISOLATION.—The ground plant material, *S. guianensis* (voucher stored at Instituto Nacional de Pesquisas da Amazonia, Manaus, AM, Brasil) (2400 g of trunk bark), collected near Manaus in December 1982, was extracted with 80% EtOH in a semi-industrial Soxhlet apparatus. Evaporation of the solvent yielded 42.4 g (1.76%) of extractables. Five years later, close to the same site, a second collection of trunk bark weighing 28.5 kg was made, and it was similarly extracted to yield 340 g of extract (1.19%). The yields of pure substances isolated and reported do not reflect the total concentration of the substances in the extract. Rather, these yields are of pure substances that were sufficient for characterization of the structures. No attempts were made to isolate the total quantity of any single substance from the extract and determine its true yield.

FRACTIONATION ACCORDING TO SCHEME 1.—Fractionation of the EtOH extract into strong bases, weak bases, and neutral fractions was as follows. A 100-g portion of the bark extract was stirred with H_2O containing 135 g of $NaHCO_3$ (pH 11.0) for 24 h. The "free bases" were extracted exhaustively with $CHCl_3$ in a continuous liquid-liquid extractor to yield, after evaporation of the $CHCl_3$ phase in vacuo at 50°, 23.10 g (23.1%) of a dark gummy material.

The aqueous fraction was filtered, and the filtrate was extracted with *n*-BuOH in a continous liquid-liquid extractor, to yield, after the *n*-BuOH was evaporated under reduced pressure, 13 g (13%; *n*-BuOH#1). The aqueous fraction was then neutralized to pH 7 with 10% aqueous HCl and re-extracted with *n*-BuOH.

Evaporation of the butanol phase gave 11 g of solid material (11%; *n*-BuOH#2). The remaining aqueous portion was discarded.

The precipitate from the initial filtration of the aqueous fraction was re-extracted with 1 liter of H_2O containing 100 g $NaHCO_3$ for 24 h. The resultant aqueous phase was extracted with $CHCl_3$ as already described and gave an additional 3 g of free bases, which were added to the previous 23.10 g.

The precipitate present in the second aqueous fraction was filtered to give 20 g of a solid material (20%; solids). The filtered aqueous fraction was then neutralized to pH 7 with 10% aqueous HCl and extracted with *n*-BuOH in the same fashion as before to give 3.8 g of extracted material (3.8%; *n*-BuOH #3). The aqueous residue was discarded.

The free bases (26.10 g) were suspended in $CHCl_3$ (1 liter) and extracted (3×200 ml) with 10% aqueous HCl. The acid fraction was carefully neutralized to pH 7 with a solution of 20% aqueous NaOH and extracted with $CHCl_3$. Evaporation of the solvent yielded 3.10 g of "strong bases" (3.1%). The remaining $CHCl_3$ fraction was evaporated onto Celite (10 g) and air-dried, and this mixture was packed into a column and eluted with 200 ml of 10% aqueous HCl. Neutralization of the acid eluent and extraction with $CHCl_3$ gave, after evaporation of the solvent, 4.75 g of "weak bases" (4.75%). The column was then washed with $CHCl_3$ to yield 14 g of material (14%). This procedure gave total recovery of 92.65%.

Results of the antimalarial bioassay showed that strong activity was present in the weak bases fraction. This was selected for further fractionation.

A portion of weak bases (50 mg) was separated by preparative tlc (2 mm plate) using 10% MeOH in $CHCl_3$ as eluent; zones were identified under uv and scraped and eluted with MeOH-$CHCl_3$ (1:3) to yield 13 fractions. Fraction F7 exhibited very potent in vitro antimalarial activity.

A portion of the weak bases fraction (2 g) was fractionated over Silica (100 g, grade H) (14). A modest pressure was necessary to provide a convenient flow rate. Initially $CHCl_3$ (300 ml) was used as eluent, followed by 2% MeOH in $CHCl_3$ (1 liter), 5% MeOH in $CHCl_3$ (500 ml), and finally 10% MeOH in $CHCl_3$ (400 ml). One hundred and ten fractions of 20 ml each were collected; after comparison by tlc, the fractions were pooled to yield 15 fractions.

The 8th fraction was similar to fraction F7 by tlc analysis. Refractionation of the 8th fraction (380 mg) using circular centrifugal chromatography (Chromatotron®) with 20% EtOAc in toluene as eluent yielded 60 mg of a substance homogeneous by tlc, **2**, and 56 mg of a second compound also homogeneous by tlc, **1**.

Gutolactone [**1**].—Compound **1** was recrystallized from Me_2CO to pale yellow platelets (56 mg): mp 182–183°; $[\alpha]^{25}D$ +49.6 (c=2.8 in MeOH); uv (MeOH) λ max (log ε) 222 (4.31); ir (KBr) ν max 3450, 2960, 2940, 2880, 1750, 1730, 1660, 1440, 1380, 1350, 1260, 1220, 1140, 1070, 1030, 980, 960, 900, 860, 820, 750 cm^{-1}; eims *m/z* (rel. int. %) $[M-99]^+$ 377 (1), 376 (1), 358 (4), 340 (2), 301 (5), 165 (16), 151 (14), 135 (26), 123 (20), 100 (15), 95 (17), 83 (100); hrms found 476.2045 (calcd for $C_{25}H_{32}O_9$, 476.2044); ^1H nmr see Table 4; ^{13}C nmr see Table 5.

Simalikalactone D [**2**].—Crystallization of **2** from Me_2CO gave white platelets, 60 mg, mp 230°. Comparison of its chromatographic behavior, mp, uv, ir spectra, and ^1H- and ^{13}C-nmr spectral data with those of an authentic sample (5) of simalikalactone D confirmed the structure of **2**.

11,12-Diacetylsimalikalactone D [**3**].—Simalikalactone D (20 mg) was dissolved in a mixture of Ac_2O (6 ml) and pyridine (4 ml), and the mixture was allowed to stir at room temperature overnight. The solvent was removed in vacuo and the residue recrystallized from $CHCl_3$ to yield **3** as white platelets, 20 mg (80%): mp 285–287° [lit (9) 282–285°]; uv (EtOH) λ max (log ε) 236 (4.27); ir (KBr) ν max 3520, 2960, 1750, 1730, 1670, 1630, 1390, 1380, 1240, 1150, 1070, 1030, 1000, 950, 840, 800, 720 cm^{-1}; eims *m/z* (rel. int. %) $[M-102]^+$ 502 (2), 501 (2), 459 (31), 442 (3), 358 (29), 301 (66), 215 (23), 207 (23), 165 (38), 159 (29), 147 (23), 142 (25), 95 (73), 91 (36), 87 (30), 85 (100); ^{13}C nmr see Table 5.

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