Merilactone, an Unusual C_{19} Metabolite from the Root Extract of *Chiococca alba*

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A C_{19} metabolite has been isolated from the methanol extract of the roots of *Chiococca alba* and characterized as a new nor-*seco*-pimarane, to which we have given the trivial name merilactone (**1**). The structure and relative stereochemistry of **1** was established by spectral data interpretation.

We have described previously the isolation of a bioactive *ent*-kaurane from the MeOH extract of the roots of *Chiococca alba* (L.) A. Hitchc. (Rubiaceae),[1] a plant commonly known as "t'uncché" or "kanchakché" by the people of the Yucatan peninsula.[2−5] The root decoction of *C. alba* is used in Yucatecan traditional medicine to cure dysentery, as a diuretic and cathartic, and to treat snake bites.[6] The leaf infusion is used to alleviate a number of ailments such as asthma, headaches, and diarrhea, and a decoction of the whole plant is reported to be effective as a laxative and against gonorrhea, skin infections, and rheumatism.[2,4] To date, the existing phytochemical knowledge of *C. alba* is limited. Two biologically active quinoline alkaloids[7] and a new oleanane-type triterpene[8] have been isolated from the root extract of the plant, while two new keto alcohols have been reported from the leaf extract.[9] In our continuing studies on the components of the root extract of *C. alba*, we wish to report herein the isolation and characterization of an unusual C_{19} metabolite (**1**).

Fractionation of the MeOH crude extract, using both an initial solvent partition procedure and successive chromatographic purifications, yielded a fraction showing a single component by TLC and GC/MS. Both the HREIMS and the positive CIMS of the purified metabolite supported a molecular formula $C_{19}H_{28}O_3$. Its IR spectrum revealed bands for two carbonyl functionalities but no hydroxyl groups, strongly suggesting that the three oxygen atoms of the molecular formula were part of a ketone and either an ester or lactone moiety. The ^{13}C NMR spectrum (Table 1) of the new product showed resonances accounting for all 28 protons in the form of four methyl groups, six methylenes, including one sp^2, and four methines, including one sp^2 and one oxymethine. Two of the remaining five quaternary carbons could be attributed to a simple carbonyl and an ester or lactone carbonyl. Another two quaternary carbons were not immediately visible but on careful analysis of the ^{13}C NMR spectrum were revealed as being of chemical shift values identical to the methylene signals at 37.5 and 36.8 ppm.

The ^1H NMR spectrum of **1** exhibited a number of discrete resonances, which included four methyl groups bonded to quaternary carbons, one of which showed a characteristic chemical shift for an acetyl methyl group, and three isolated ABX spin systems, attributable in turn to C−CH=CH_2, C−CH(O)-CH_2−C, and C−CH−CH_2. Unfortunately, several of the remaining protons were associated with two unresolvable multiplets at δ 1.60 and 1.40. Direct C−H bonds were identified by means of an HC−COBI experiment and long-range heteronuclear interactions from the HMBC spectrum. The ^2J and ^3J couplings observed from the latter are listed in Table 1. The ethenyl substituent was shown to share a quaternary carbon with one of the methyl groups giving rise to a partial structure **a** (Figure 1). Likewise, the acetyl group shared a quaternary carbon with a second methyl group, and this fragment of the structure could be expanded through observed HMBC interactions of the methine proton at δ 2.32 (H-10) with C-1 (^2J), C-9(^2J), and C-11 (^3J), and of the methyl group protons at δ 0.89 (H-20) with C-10 (^3J), C-8 (^3J), and C-11 (^3J). This permitted the identification of a second partial structure **b** (Figure 1), which incorporated the lactone ring and the final methine. A ^3J interaction between the methylene protons of the C−CH(O)−CH_2−C spin system and the methyl (C-17) and methine (C-15) carbons of fragment **a** linked the two partial structures and left unaccounted only two methylenes. As the proton at δ 1.81, which was already placed as part of the methylene bound to the C(Me)COMe in partial structure **b**, showed

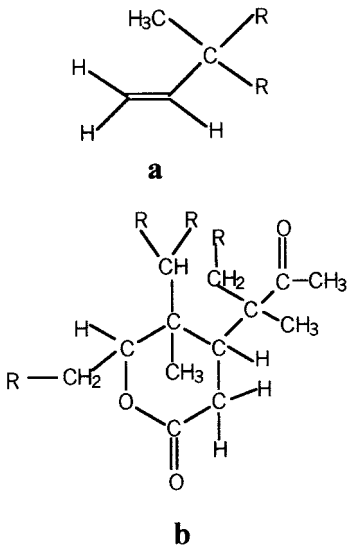

Figure 1. Partial structures of merilactone (**1**).

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Table 1. NMR Data for Merilactone (**1**)[a]

position	^{13}C	^{1}H	HMBC correlations	NOESY correlations
1ax	30.5	2.42 dd (18.3, 12.6)	36.8. 44.1, 170.9, 50.1	Me-19
1eq		2.67 dd (18.3, 6.2)		
2	170.9			
3	25.5	2.14 s	212.6	H-10
4	212.6			
5	50.1			
6eq	36.8	1.81 ddd (9.6, 3.0, 2.0)	39.9, 44.1	
6ax		1.60 m		
7	23.4	1.60 m		
		1.40 m		
8	39.9	1.40 m		H-11
9	36.8			
10	44.1	2.32 dd (12.6, 6.1)	8.4, 16.9, 30.5, 36.8, 50.1, 85.0	H-11, Me-3
11	85.0	4.22 dd (12.0, 4.8)	8.4, 44.1	H-10, H-8, H-12_{eq}, Me-17
12ax	36.3	1.73 bdd (12.6, 12.6)	25.0, 37.5, 149.0	Me-20
12eq		1.62 ddd (13.6, 4.7, 1.5)		H-11
13	37.5			
14eq	37.5	1.10 dd (12.0, 1.5)		H-15
14ax		1.40 m		
15	149.0	5.80 dd (17.4, 10.7)	37.5	H-14_{eq}
16	110.1	4.96 dd (17.4, 0.8)	37.5, 149.0	
		4.92 dd (10.7, 0.8)		
17	25.0	1.12 s	37.5, 149.0	H-11
19	16.9	1.27 s	36.8, 44.1, 50.1, 212.6	H-1_{ax}, Me-20
20	8.4	0.89 s	36.8, 39.9, 44.1, 85.0	H-12_{ax}, Me-19

[a] ^{1}H and ^{13}C NMR spectra were run in $CDCl_3$ at 400 and 100 MHz, respectively. Chemical shift values are given in parts per million (ppm) relative to the solvent signal (7.26 and 77.0 ppm for ^{1}H and ^{13}C NMR spectra, respectively); coupling constant values are given in hertz.

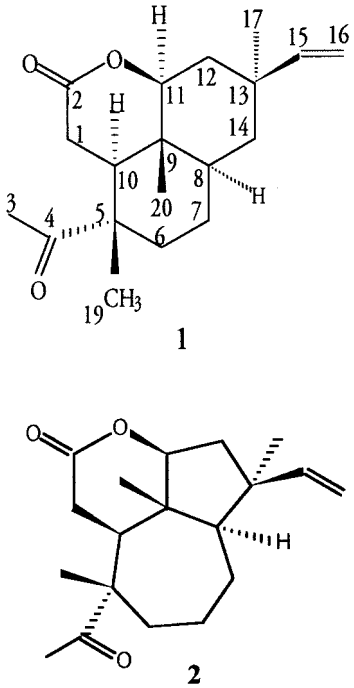

Figure 2. Possible structures for merilactone (**1**).

coupling requiring its placement adjacent to another methylene, two structures were possible, **1** and **2** (Figure 2).

Structure **1** was chosen for the following reasons. First, all observable $^{1}H-^{1}H$ couplings supported normal six-membered-ring conformations. Second, the HMBC results indicated a ^{3}J correlation between one of the H-12 methylene protons (1.73 ppm) and C-14 (37.5 ppm) and between H-15 (5.80 ppm) and C-14 (37.5 ppm), correlations that are more likely in structure **1** than **2**. Furthermore, the NOESY experiment clearly placed the δ 1.10 proton (H_{eq}-14), which is part of a methylene, adjacent to the CH_2=CHC(Me) component of the molecule, an interaction that is more likely in **1** than **2**. Full analysis of the NOESY spectrum

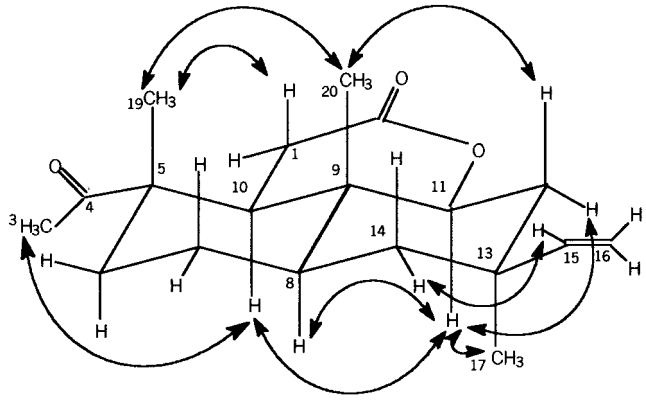

Figure 3. NOESY correlations of merilactone (**1**).

(Table 1, Figure 3) revealed the relative stereochemistry of 4-acetyl-4,8,9b-trimethyl-8-vinyldecahydrobenzo[*de*]chromen-2-one as depicted in structure **1**. We have given **1** the trivial name merilactone.

The biogenetic origin of **1** is obscure. Given that *C. alba* is known to biosynthesize diterpenes, it seems most probable that **1** is a nor-diterpene that has undergone a ring fission and subsequent lactonization. One plausible scenario would be an origin as a pimar-9(11),15-diene (**3**), which, via oxidation at C-11, undergoes a series of 1,2-migrations to give the intermediate **4**. Oxidative elimination of the C-4 exomethylene and cleavage between C-2 and C-3 (**5**) with subsequent cyclization between a carboxylic acid originating from C-2 and the C-11 hydroxyl results in structure **1** (Figure 4).

Experimental Section

General Experimental Procedures. The melting point was determined with an Electrothermal type 1A6304 melting point apparatus and is uncorrected. The optical rotation was measured using a JASCO DIP360 polarimeter, and the IR spectrum was recorded in $CHCl_3$ in a Perkin-Elmer 683 spectrophotometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were obtained in $CDCl_3$ on a Varian Unity 400

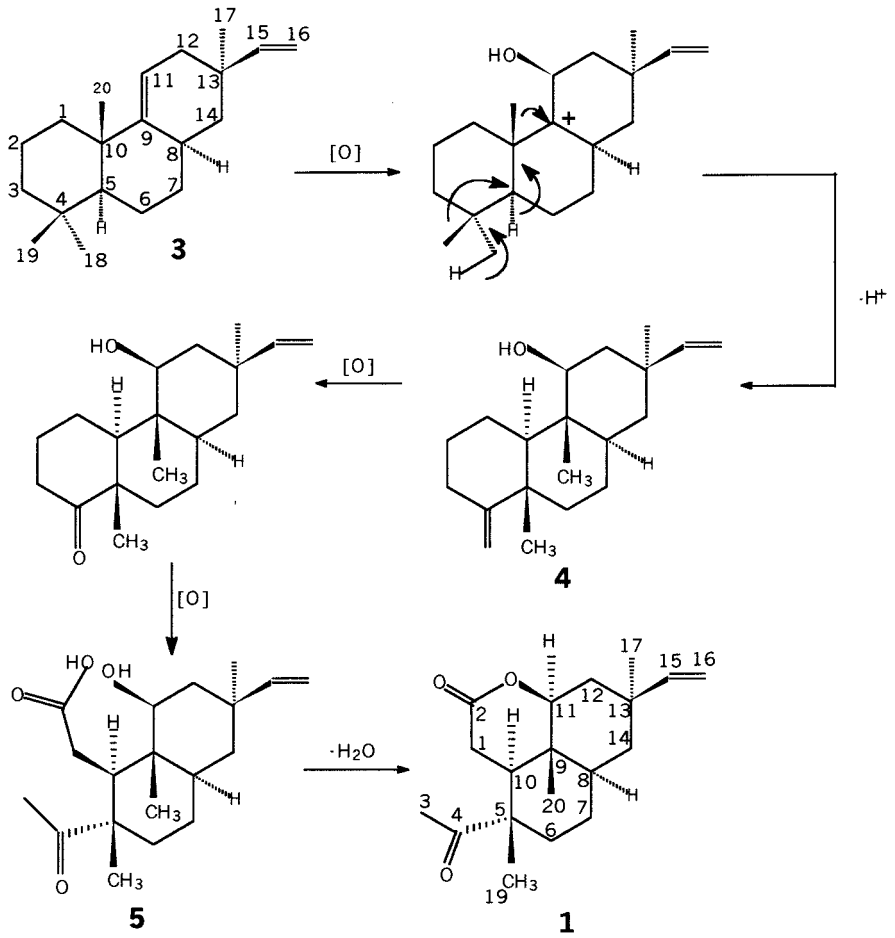

Figure 4. Possible biosynthetic origin of merilactone (**1**).

spectrometer using the $CHCl_3$ signal (7.26 and 77.00 ppm, respectively) as reference. HREIMS and CIMS were recorded at 70 eV on Kratos AEI MS-50 and AEI MS-12 mass spectrometers, respectively. GC/MS was performed on a Hewlett-Packard 5890 gas chromatograph [1.0 μL of 3% sample in $CHCl_3$, HP Ultra 2 column (cross-linked 5% Ph Me silicone, 25 m length × 0.32 mm i.d. × 0.52 μm film thickness), flow rate 1 mL/min, temperature program: T_1 150 °C, T_2 300 °C, gradient 10 °C/min, injector temperature 300 °C, detector temperature 290 °C] coupled to a Hewlett-Packard 5971A mass selective detector. Analytical and preparative TLC were carried out using precoated Si gel aluminum and glass plates, respectively (E. M. Merck, DC Alufolien, Kieselgel 60 F254, 0.20 mm thickness, and E. M. Merck, DC Fertigplatten, Kieselgel 60 F_{254}, 0.25 mm thickness). Chromatograms were examined under UV light in a UV-viewing cabinet (Spectroline Model CX-20) and visualized by dipping in 4% phosphomolybdic acid solution containing a trace of ceric sulfate in 5% sulfuric acid, followed by drying and gentle heating. Medium-pressure column chromatography (flash) purifications were run according to Still et al.[10] using Si gel 60 (230−400 mesh) from Aldrich Chemical Co.

Plant Material. The roots of *C. alba* were collected during July 1992 from plants growing in a field located at km 24 of the Merida-Progreso highway in Yucatán, Mexico. A voucher specimen has been deposited in the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán (CICY) under the collection number 696. The plant material was washed with tap water and dried, first for a week at room temperature, and then for 72 h in an oven at 55 °C. The dried roots were ground using a Brabender Dusiburg (880804 type) mill and a No. 2 sieve.

Extraction and Isolation. Soxhlet extraction of the ground plant material (195.12 g) using MeOH (2 L) produced the crude MeOH extract (16.01 g), which was suspended in 1250 mL of a 3:2 mixture of H_2O−MeOH. The resulting suspension was partitioned successively between hexane, CH_2Cl_2, and EtOAc to yield low-polarity (0.31 g), medium-low-polarity (2.45 g), and medium-polarity fractions (1.19 g), respectively. Succesive flash column chromatography (CH_2Cl_2−acetone, 98:2) and preparative TLC (eluted three times, hexane−acetone, 80:20) purifications of the low-polarity fraction yielded 7.8 mg of **1** in pure form.

Merilactone (1): amorphous solid; mp 124−126 °C; $[\alpha]_D$ +21.1° (*c* 0.9 MeOH); t_R 3.9 min; R_f 0.44 [hexane−acetone (8:2), 2×]; IR ν_{max}($CHCl_3$) 3020 (CH stretch, olefinic), 1725 (C=O stretch, lactone), 1700 (C=O stretch, ketone) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; CIMS (NH_3) *m/z* 322 [M^+ + 18] (34), 305 [M^+ + 1] (100); HREIMS *m/z* 304.2039 [M^+] (11) (calcd for $C_{19}H_{28}O_3$: 304.2038, Δ −0.0001 mmu), 261 [M^+ − $COCH_3$] (100).

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