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# KETOALCOHOLS, LIGNANS AND COUMARINS FROM *CHIOCOCCA ALBA*

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**Key Word Index**—*Chiococca alba*; Rubiaceae; leaves; 4-hydroxyheptadecan-7-one; 5-hydroxyoctadecan-11-one; 5,7,4'-trimethoxy-4-phenylcoumarin; exostemin; matairesinol; D-mannitol.

**Abstract**—Two new hydroxyketones, 4-hydroxyheptadecan-7-one and 5-hydroxyoctadecan-11-one, along with 5,7,4'-trimethoxy-4-phenylcoumarin, exostemin, matairesinol and D-mannitol have been isolated from leaves of *Chiococca alba* and identified by spectral data and chemical studies.

## INTRODUCTION

The genus *Chiococca* (Rubiaceae) includes 22 species distributed in America, the West Indies, Mexico and Venezuela. The roots of *C. alba* are reported to be used in folk medicine as a tonic, for ganglion inflammation, a diuretic, an antivirus, an antioedema and an aphrodisiac [1]; the closely related species (*C. brachiata*) is mentioned in the French Pharmacopeia [2].

Neither chemical nor pharmacological studies of the constituents of this genus have been reported. We have now carried out a chemical investigation of the leaves of *C. alba*. Our examination of a leaf-extract has revealed the absence of alkaloids, but the presence of coumarins and lignans. We report the isolation and structure elucidation of two new hydroxyketones, 4-hydroxyheptadecan-7-one (**1**) and 5-hydroxyoctadecan-11-one (**2**), as well as the known compounds, 5,7,4'-trimethoxy-4-phenylcoumarin (**3**), exostemin (**4**) matairesinol (**5**) and D-mannitol (**6**) which have not been previously isolated from the genus *Chiococca*. We also report $^{13}C$ NMR spectral data for exostemin and matairesinol.

## RESULTS AND DISCUSSION

Silica gel column chromatography of a *n*-hexane extract of the leaves afforded 4-hydroxyheptadecan-7-one (**1**) and 5-hydroxyoctadecan-11-one (**2**) and that of an ether extract furnished 5,7,4'-trimethoxy-4-phenylcoumarin (**3**), exostemin (**4**) and matairesinol (**5**).

Compound **1**, gave a positive 2,4-dinitrophenylhydrazine test and showed IR absorption bands for a hydroxyl group at 3450 $cm^{-1}$ and a carbonyl group at 1720 $cm^{-1}$. Its long chain nature was revealed by the presence of bands of 2920, 2850 and 715 $cm^{-1}$. Its molecular formula $C_{17}H_{34}O_2$ was assigned from the HR mass spectrum which showed a $[M]^+$ at *m/z* 270.18031. The appearance of two series of peaks, *m/z* 41, 55, 69 and 43, 57, 71, 85 with fragments differing by 14 mass units suggested the presence of a long straight methylene chain [3]; this was further supported by the absence of an $[M-15]^+$ peak [4]. In addition, the presence of an $[M+1]^+$ peak is characteristic of an unsymmetrical ketone [5, 6]. The location of the carbonyl group at C-7 was deduced from the prominent $\alpha$-fission ions at *m/z* 101, 169, 129, 143 and $\beta$-fission ions, involving McLafferty rearrangement, at *m/z* 144 and 184 [3]; the ion at *m/z* 58 is obtained by double rearrangement, and is characteristic of a ketone having a $\gamma$-H in both alkyl fragments. The base peak at *m/z* 74 and the significant ions at *m/z* 43, 227 and 196 are due to the $\alpha$-fission of the hydroxyl group, which is thus assigned to C-4. The $^1H$ NMR spectrum of **1** showed a triplet, $J=7$ Hz at $\delta$0.9 for terminal methyl groups and a broad singlet at $\delta$1.22 for 10 methylene groups. A triplet, $J=6$ Hz at $\delta$2.20 is indicative of methylene groups adjacent to the carbonyl group. The signal at $\delta$1.60 was due to methylene groups $\beta$ to the carbonyl function. A broad singlet at $\delta$3.26 was assigned to CHOH proton was observed at $\delta$2.43. Jones oxidation of **1** provided a diketone, mp, 72–73° with no OH absorption in its IR. The mass spectrum had a $[M]^+$ ion at *m/z* 268 ($C_{17}H_{32}O_2$). The location of the two carbonyl groups at C-4 and C-7 is supported by $\alpha$-fission ions at *m/z* 72, 225 and at 127, 169, respectively. The corresponding $\beta$-fission ions are observed at *m/z* 240, 142 and 114. The above data are best accommodated by the structure 4-hydroxyheptadecan-7-one for compound **1**. Treatment of **1** with acetic anhydride–pyridine furnished a ketoacetate, mp 64–65°, having IR bands at 1735, 1710 and 1260 $cm^{-1}$.

Compound **2**, had IR absorption bands at 3460 (OH), 2915, 2850, 1455, 730, 720 (long chain), 1720 (CO) and 1380 $cm^{-1}$ and also gave a positive 2,4-dinitrophenylhydrazine test. Its molecular formula $C_{18}H_{36}O_2$ was determined from the HR mass spectrum which showed a $[M]^+$ at *m/z* 284.16219. The long chain asymmetrical nature of the ketone was indicated by the presence of $[M+1]^+$ and the absence of $[M-15]^+$ ions in the mass spectrum [4–6]. The long chain nature of the compound was also supported by two series of fragments with a uniform loss

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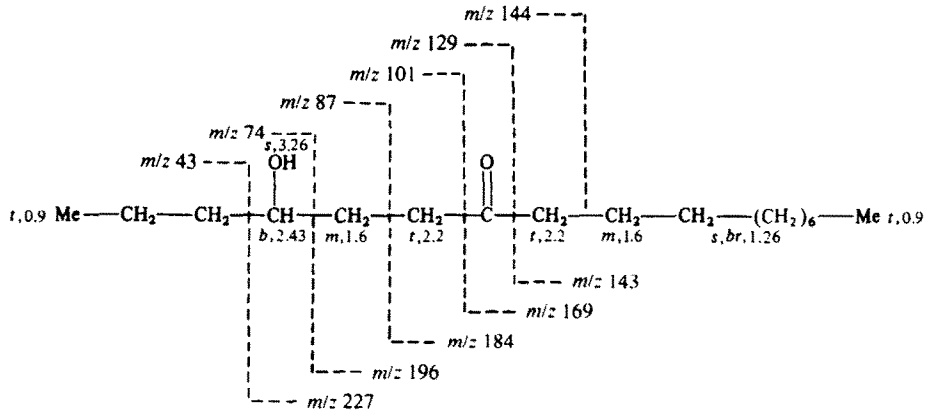


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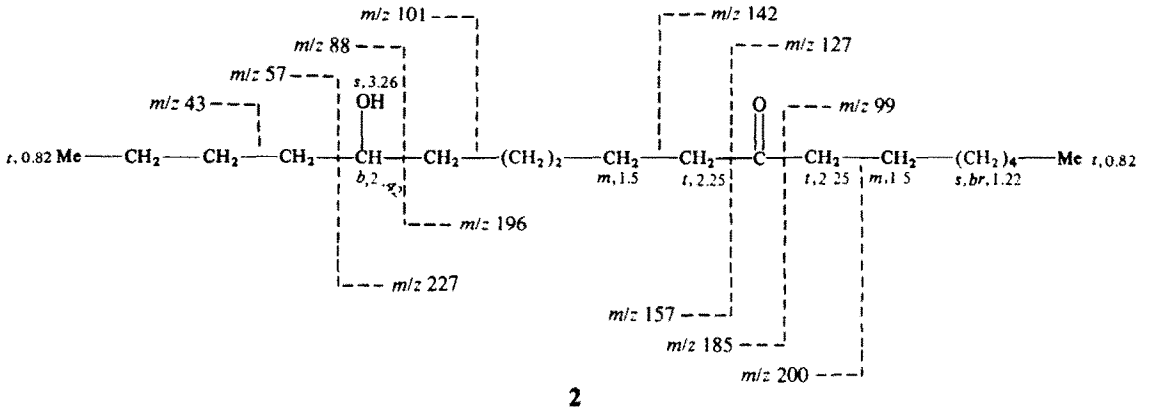


2

of 14 mass units [3]. The position of the carbonyl group at C-11 was indicated by α-fission fragments at *m/z* 157, 127, 185, 99 and β-fission fragments, involving Mclafferty rearrangement, at *m/z* 142 and 200 [3]. A significant ion at *m/z* 58 is due to double rearrangement. The location of the hydroxyl group at C-5 is supported by significant α-fission ions at *m/z* 57, 227 and 88 (base peak). The $^1$H NMR spectrum showed signals for terminal methyl groups at δ0.82 (6H, *t*, $H_3$-1, $H_3$-18, $J = 6$ Hz) and 10 methylene groups at δ 1.22 (20H, *br s* $(CH_2)_{10}$]. A triplet at δ2.25 is indicative of four protons of the two methylene groups adjacent to the carbonyl group. The signal at δ1.50 (4H, *m*, $H_2$-9, $H_2$-13) is due to methylene groups β to the carbonyl function. A broad singlet at δ3.26 was observed for the CHOH proton, whereas the CHOH proton resonated at δ2.42. Oxidation of **2** with Jones reagent afforded a diketone, mp 75–76°, with IR bands at 1720 (sh), 1700 (CO) and no absorption band corresponding to a hydroxyl group. The mass spectrum had a $[M]^+$ at *m/z* 282, suggesting the molecular formula $C_{18}H_{34}O_2$. The location of the two carbonyl groups at C-5 and C-11 was confirmed by α-fission ions at *m/z* 86, 225 and at 127, 183, respectively. The corresponding β-fission ions were observed at *m/z* 240, 198 and 156. These data are in full agreement with the structure 5-hydroxyoctadecan-11-one for **2**. Treatment of **2** with acetic anhydride–pyridine afforded a ketoacetate, mp 66–67°, having IR bands at 1730, 1700 and 1255 $cm^{-1}$.

The two coumarins $C_{18}H_{16}O_5$ (**3**) and $C_{18}H_{16}O_6$ (**4**) showed analogous UV [7] and IR spectra. Their physical properties, UV, IR, mass and $^1$H NMR spectra are in full agreement with those reported for 5,7,4′-trimethoxy-4-phenylcoumarin and 8-hydroxy-5,7,4′-trimethoxy-4-phenylcoumarin (exostemin), respectively [8, 9]. Acetylation and methylation of **3** afforded a derivatives devoid of hydroxyl absorption and its $^{13}$C NMR spectral data [$CDCl_3$, 200 MHz, δ160.6 (C-2), 160.4 (C-4′), 156.7 (C-4), 151.7 (C-8), 150.6 (C-6), 143.7 (C-9), 132.6 (C-1′), 129.5 (C-2′ and C-6′), 128.3 (C-5), 113.6 (C-3′ and C-5′), 113.5 (C-3), 104.5 (C-10), 94.1 (C-7), 57.2 (C-11), 57.1 (C-12) and 56.1 (C-13)] are reported here for the first time.

Compound **5** was obtained as powder from ethanol. Its molecular formula $C_{20}H_{22}O_6$ was assigned from the HR mass spectrum, which showed a $[M]^+$ at *m/z* 358.141638. It was identified as matairesinol by comparison with published data [10, 11]. $^{13}$C NMR spectral data [$CDCl_3$, 200 MHz, δ178.85 (C-9′), 146.62 (C-3, C-3′), 144.24 (C-4, C-4′), 129.67 (C-1, C-1′), 121.92 (C-6, C-6′), 114.31 (C-2, C-2′), 111.41 (C-5, C-5′), 71.54 (C-8′), 55.66 (C-9), 46.41 (C-8), 40.86 (OMe), 39.13 (C-7′) and 34.43 (C-7)] are again reported here for the first time. Matairesinol has been reported previously from *Trachelospermum asiaticum* var. *intermedium* [10], although this is the first report of its isolation from *Chiococca*.

Compound **6** was obtained as prisms from ethanol, mp 166°, identified as D-mannitol by direct comparison with

authentic sample and spectral studies [12]. Detection of the compound substantiates the earlier observation [12] of the occurrence of this sugar in the Rubiaceae.

## EXPERIMENTAL

Mps: uncorr. IR were recorded in KBr. $^1$H NMR (200 MHz) were determined in $CDCl_3$ using TMS as int. std. MS were recorded at 70 eV using a direct inlet system. Sepns by CC utilized silica gel (35–70 mesh, Merck). TLC was performed on a precoated silica gel layers and visualized either by exposure to $I_2$ vapour, or spraying with 2,4-dinitrophenylhydrazine.

*Plant material.* Leaves of *C. alba* L. were collected and identified by the Department of Botany of the Botanical Garden of Santo Domingo, Dominican Republic.

*Extraction and isolation of compounds.* Air-dried powdered leaves (0.75 kg) were extracted in a Soxhlet with *n*-hexane for 20 hr. The dried defatted plant material was subsequently exhaustively extracted by percolation with EtOH. The concd EtOH extract (300 ml) was left overnight at 5° when a greenish substance pptd. This was filtered off, purified by repeated crystallization from boiling EtOH, when prismatic crystals (892 mg) were obtained, mp 166° corresponding to that of D-mannitol (**6**) [12]. Its identity was confirmed by mmp, mp of acetate, IR, NMR and MS. The filtrate was concd (19 g) and partitioned between $Et_2O$ and $H_2O$ after removal of EtOH. The concd $Et_2O$ extract (6.12 g) was passed through a column of silica gel (400 g) with $CHCl_3$–MeOH mixts. Extended CC and crystallization gave pure compounds **3**–**5**. The *n*-hexane extract was evapd *in vacuo* to give 8.22 g which was subjected to CC on silica gel (500 g) with *n*-hexane to give compounds **1** and **2**.

*4-Hydroxyheptadecan-7-one* (**1**). Removal of hexane afforded a residue (68 mg), mp 67–68° (EtOH). IR $\nu_{max}$ cm$^{-1}$: 3450, 2920, 2850, 1720, 1460, 1390, 725, 715. MS *m/z* (rel. int.): 270 ([M]$^+$, $C_{17}H_{34}O_2$, 17), 227 (10), 213 (2), 199 (3), 196 (2), 184 (4), 169 (3), 157 (2), 144 (3), 143 (13), 141 (2), 129 (6), 101 (5), 87 (62), 85 (4), 75 (18), 74 (100), 71 (6), 69 (13), 58 (3), 57 (16), 55 (23), 43 (27), 41 (24). $^1$H NMR ($CDCl_3$), see Fig. 1. Compound **1** (18 mg) was treated with pyridine (1 ml) and $Ac_2O$ (1 ml) overnight at room temp. when worked-up it afforded a residue, mp 64–65° ($Me_2CO$), IR $\nu_{max}$ (cm$^{-1}$): 2925, 2860, 1735, 1710, 1455, 1365, 1260, 725 and 715.

*Jones oxidation of* **1**. Compound **1** (40 mg) was dissolved in $Me_2CO$ (200 ml) and 7 N chromic acid added dropwise with constant shaking. Completion of the reaction was indicated by persistence of a yellow colour in the supernatant liquid for at least 10 min. Solvent was concd to 30 ml *in vacuo*, the concentrate dil with $H_2O$ (80 ml) and extracted with $Et_2O$ (4 × 50 ml). The $Et_2O$ extract was washed with $H_2O$ (2 × 40 ml) and dried ($Na_2SO_4$). Removal of solvent furnished a residue, mp 72–73° ($Me_2CO$–MeOH). IR $\nu_{max}$ cm$^{-1}$: 2910, 2840, 1720, 1700, 1450, 730 and 720. MS *m/z* (rel. int.): 268 (2), 240 (2), 225 (3), 142 (5), 127 (22), 114 (13), 72 (55) and 43 (100).

*5-Hydroxyoctadecane-11-one* (**2**). Removal of hexane afforded a residue (60 mg), mp 69–70° (MeOH). IR $\nu_{max}$ cm$^{-1}$: 3460, 2915, 2850, 1720, 1455, 1380, 730 and 720. MS *m/z* (rel. int.): 284 ([M]$^+$, $C_{18}H_{36}O_2$, 20), 241 (12), 227 (2), 213 (4), 200 (4), 196 (1), 185 (4), 157 (15), 142 (7), 129 (9), 127 (2), 115 (7), 101 (55), 89 (18), 88 (100), 85 (5), 83 (9), 71 (9), 69 (14), 57 (18), 55 (22), 43 (30) and 41 (22). $^1$H NMR ($CDCl_3$) see Fig. 2. Compound **2** (12 mg) was treated with pyridine (1 ml) and $Ac_2O$ (1 ml) overnight at room temp. After work-up it furnished a residue, mp 66–67° ($Me_2CO$). IR $\nu_{max}$ cm$^{-1}$: 2920, 2850, 1730, 1700, 1455, 1370, 1255, 725 and 715. Oxidation of **2** with Jones reagent as described above afforded a diketone, mp 75–76°. IR $\nu_{max}$ cm$^{-1}$: 2900, 2830, 1720, 1700, 1440, 726 and 700. MS *m/z* (rel. int.): 282 (2), 264 (2), 240 (3), 225 (5), 198 (6), 183 (12), 156 (17), 127 (22), 99 (25), 86 (13), 57 (100) and 43 (35).

*5,7,4′-Trimethoxy-4-phenylcoumarin* (**3**). Amorphous powder (8.7 mg), $C_{18}H_{16}O_5$, mp 152° (EtOH). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 260 (4.06), 315 (4.25). $^1$H NMR ($CDCl_3$): δ7.25 (2H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.93 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.53 (1H, *d*, *J* = 2.5 Hz, H-8), 6.24 (1H, *d*, *J* = 2.5 Hz, H-6), 5.90 (1H, *s*, H-3), 3.87 (6H, *s*, OMe-7, Me-4′), 3.48 (3H, *s*, OMe-5). IR $\nu_{max}$ cm$^{-1}$: 1710, 1625, 1590, 1510, 1160, 1110, 1060, 950, 872, 860, 840. MS *m/z* (rel. int.) 312 [M]$^+$ (100), 284 [M − CO]$^+$ (51), 269 [M − MeCO]$^+$ (48), 241 [M − 43 − CO]$^+$ (2).

*8-Hydroxy-5,7,4′-trimethoxy-4-phenylcoumarin (exostemin)* (**4**). Pale yellow crystals (35 mg), $C_{18}H_{16}O_6$, mp 174° (EtOH). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 260 (4.06), 315 (4.26). $^1$H NMR ($CDCl_3$): δ7.24 (2 H, *d*, *J* = 8.5 Hz, H-2′, H-6′), 6.92 (2H, *d*, *J* = 8.5 Hz, H-3′, H-5′), 6.34 (1H, *s*, H-6), 5.80 (1H, *s*, H-3), 5.75 (OH, *br s*, OH-8), 3.98 (3H, *s*, OMe-7), 3.87 (3H, *s*, OMe-4′), 3.46 (3H, *s*, OMe-5). IR $\nu_{max}$ cm$^{-1}$: 3450, (free OH), 1710, (α-pyrone C=O), 1590, 1515, 1170, 1110, 1070, 950, 875, 865, 845. MS *m/z* (rel. int.) 328 (100), 312 (40), 299 (24), 284 (38), 269 (6), 257 (10), 201 (7).

*Exostamin acetate.* Treatment of **2** (12 mg) with pyridine (1 ml) and $Ac_2O$ (1 ml) and refluxing for 1 hr, afforded a residue which crystallized from MeOH to give exostamin acetate, mp 203–205°. IR $\nu_{max}$ cm$^{-1}$: 1770, 1725. $^1$H NMR ($CDCl_3$): δ2.35 (3H, *s*, acetyl group).

*O-Methylexostamin.* Refluxing of **3** (10 mg) with DMSO (1 ml) in 8 ml dry $Me_2CO$ and $K_2CO_3$ (2.5 ml), furnished crystals of *O*-methylexostamin, mp 143–145°. IR $\nu_{max}$ cm$^{-1}$: 1722 (ester group). $^1$H NMR ($CDCl_3$), δ3.35, 3.91, 3.95 and 3.97 (four methoxyl groups).

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