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NORNEOLIGNAN AND PHENOLS FROM *CURCULIGO CAPITULATA*

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Key Word Index—*Curculigo capitulata*; Amaryllidaceae; rhizome; 2,4-dichloro-5-methoxy-3-methyl-phenol; curlignan; 4-ethoxy-3-hydroxymethyl-phenol.

Abstract—Nine compounds were isolated from the chloroform-soluble fraction of *Curculigo capitulata*. Of these, 2,4-dichloro-5-methoxy-3-methylphenol, curlignan and 4-ethoxy-3-hydroxymethylphenol are new natural products. Their structures were elucidated mainly based on spectral analysis. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Recent chemical investigation of *Curculigo capitulata* (Lour.) O. Kuntze alias *C. recurvata*, has led to the isolation of a series of norlignan glycosides [1–4] and curcapitoside [3], a novel glucosyl-fused phenanthropyran, from the polar fraction. Three of the norlignan glucosides, nyasicoside, 1*S*-*O*-butyl- and 1*R*-*O*-butyl-nyasicosides, were found to possess anti-arrhythmic properties [4]. Being interested in such biological activity, we attempted to obtain potential norlignan aglycones from the less polar fraction of this species, which has not been explored yet. Here, we report the outcome of this study.

RESULTS AND DISCUSSION

The chloroform soluble fraction of the ethanol extract of the rhizome was subjected to repeated column chromatography and yielded nine compounds. Six of them were identified as vanillin, 4-hydroxybenzaldehyde [5], ethyl protocatechuate [6], orcinol-1-*O*-*β*-D-glucoside [7], 2,6-dimethoxy-benzoic acid [8] and *β*-sitosterol 3-*O*-*β*-D-glucoside [9]. Three of them, 2,4-dichloro-5-methoxy-3-methylphenol (**1**), curlignan (**2**) and 4-ethoxy-3-hydroxymethylphenol (**3**), were found to be novel natural products. Their structures were elucidated as follows.

Compound **1** was identified as 2,4-dichloro-5-methoxy-3-methylphenol by comparison of its EI mass and ^{1}H NMR spectral data [four singlets at δ 6.52 (1H), 5.59 (1H, D_2O exchangeable), 3.83 (3H, OMe) and 2.44 (3H, Ph–Me)] with reported data [10]. It had been reported as the acid hydrolysis product of curculigine A, isolated from the related plant species, *C. orchioides* [10], but it is the first natural occurrence of this compound. Its structure was confirmed by two NOE experiments which indicated that the aryl proton signal (δ 6.52) was enhanced by irradiation at the methoxyl singlet (δ 3.83) but not at the aryl methyl singlet (δ 2.44).

Compound **2**, a yellowish viscous liquid, $[\alpha]_D^{25}$ +40°C (*c* 0.5, MeOH), had a molecular formula $C_{19}H_{20}O_7$ (HR EI mass spectrometry). It is also phenolic as exemplified by the IR absorption at 3400 cm^{-1} and the bathochromic shift in the UV spectrum when measured in alkaline conditions. Its ^{1}H NMR spectrum (Me_2CO-d_6) revealed an ABX-pattern and an AB-pattern for the five aromatic protons, three MeO singlets, a double triplet (1H, δ 3.62) coupled to a doublet (1H, δ 5.65) and a multiplet (2H, δ 3.84), as identified in a COSY-45 spectrum. The ^{1}H NMR spectrum of its acetylated product (**2a**) revealed two additional singlets for acetyl groups (δ 2.28 and 2.03) and downfield shifted signals for the oxygenated methylene protons from δ 3.84 (2H, *m*) to δ 4.45 (1H, *dd*, *J* = 11.3, 5.4 Hz) and δ 4.30 (1H, *dd*, *J* = 11.3, 7.5 Hz), suggesting the presence of a hydoxymethyl group in **2**. The ^{13}C NMR data revealed that **2** contained two aryl groups (four oxygenated and three

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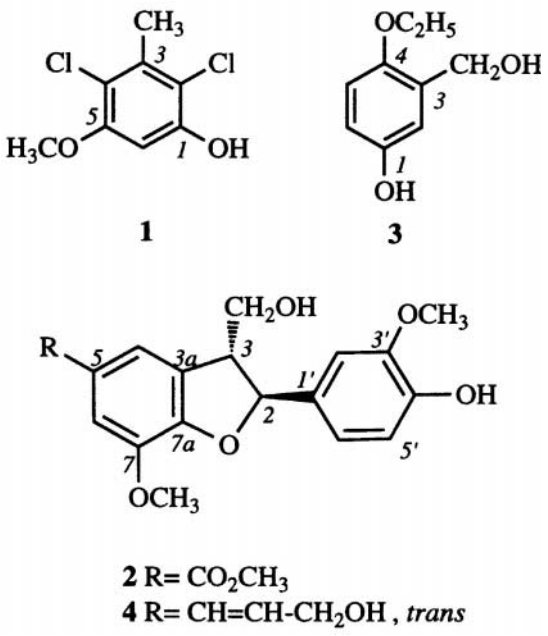

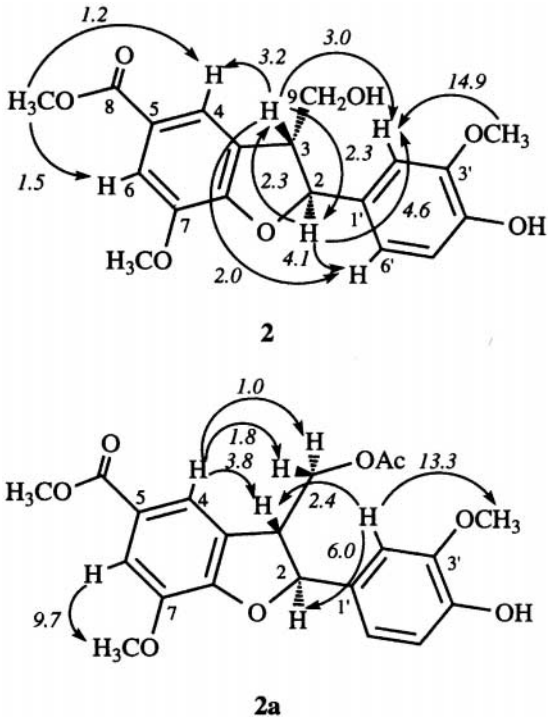

Fig. 1. NOEs (italics, %) of **2** (Me_2CO-d_6) and **2a** ($CDCl_3$).

nonoxygenated quaternary carbons and five methines), one conjugated carboxylate (δ 167.0, *s*), two oxygenated aliphatic carbons (δ 89.5, *d*; δ 64.2, t), one aliphatic methine (δ 54.1, *d*) and three methoxy, one of which is an ester methyl (δ 52.0, *q*). These data pooled together would suggest **2** to be a 2-phenyl benzofuran.

This proposed skeleton was confirmed by NOE studies of **2** and its peracetate **2a** (Fig. 1). Among these, irradiation at the H-2 doublet (δ 5.65) or H-3 double triplet (δ 3.62) caused the enhancement of the corresponding H-2′ and H-6′ signals. A chemical model study of **2** indicated that these two NOE results could be possible only if H-2 and H-3 are *trans*-oriented. The absolute stereochemistry at C-2 and C-3 of **2** was determined to 2*S*,3*R* by the comparison of its CD curve with that of 2*S*,3*R*-(+)-dehydro-diconiferyl alcohol (**4**) [11], both showing one negative and one positive Cotton effect around 230 and 280 nm, respectively. These data established the structure of **2** as depicted in Fig. 1. This novel natural product is named curlignan after its plant origin and skeleton similar to neolignan. Curlignan (**2**) could be produced biogenetically by an oxidative coupling of two coniferyl alcohol, followed by oxidative degradation of the side-chain at C-5.

The assigned structure for **2** was also confirmed by the analysis of the HMBC spectrum (Table 1). Critical correlations for the ring skeleton include the couplings of H-2 to C-2′, C-6′ and C-9, and H-3 to C-3a and C-1′. The unambiguous ^1H and ^{13}C NMR assignments for **2** are listed in Table 1 from the analysis of NOE, HMQC and HMBC spectra. The ^1H NMR spectrum measured in $CDCl_3$ was also assigned and is listed in the Experimental section for reference, although it was not so well-resolved as that measured in Me_2CO-d_6.

Compound **3**, a yellowish viscous liquid, had a molecular formula $C_9H_{12}O_3$ as deduced from EI mass spectrometry and NMR data. It is phenolic as exemplified by the IR absorption at 3300 cm^{-1} and the bathochromic shift in the UV spectrum when measured in alkaline conditions. Its ^1H NMR spectrum revealed an ABX-pattern for the aromatic protons, signals for an ethoxyl group and a two-

Table 1. ^{13}C and ^1H NMR (δ, mult.) (Me_2CO-d_6) and HMBC data for compound **2**

Position	^{13}C	^1H (*J* in Hz)	HMBC (*J* = 8 Hz) correlated C (#)
2	89.5 *d*	5.65 *d* (6.7)	2′,6′,9
3	54.1 *d*	3.62 *dt* (6.1, 6.7)	3a, 1′
3a	130.6 *s*		
4	119.8 *d*	7.61 *d* (1.3)	6, 7a, 8
5	124.1 *s*		
6	114.6 *d*	7.50 *d* (1.3)	4, 7a, 8
7	144.9 *s*		
7a	153.6 *s*		
8	167.0 *s*		
9	64.2 *t*	3.84 *m*	
1′	133.6 *s*		
2′	110.6 *d*	7.03 *d* (1.2)	2, 4′, 6′
3′	148.5 *s*		
4′	147.6 *s*		
5′	115.7 *d*	6.81 *d* (8.1)	1′, 3′
6′	120.2 *d*	6.88 *dd* (1.2, 8.1)	2, 2′, 4′
7-OMe	56.3 *q*	3.88 *s*	7
8-OMe	52.0 *q*	3.83 *s*	8
3′-Ome	56.4 *q*	3.81 *s*	3′

proton singlet (δ 4.60) corresponding to an oxygenated benzylic methylene. These data indicated **3** to be a 1,2,4-trisubstituted benzene. The location of these substituents was determined by NOE experiments. Irradiation at the methylene singlet enhanced the *meta*-coupled aromatic proton (δ 6.50, *d*, J = 3.0 Hz), and the methylene quartet (δ 3.57, J = 7.0 Hz). Irradiation at the latter quartet enhanced an *ortho*-coupled aryl proton (*d*, J = 8.6 Hz) and the methyl triplet (δ 1.24, J = 7.0 Hz), in addition to the methylene singlet. These data taken together established **3** to be 4-ethoxy-3-hydroxymethylphenol.

Besides these compounds, some cycloartenol related triterpenes, which had been found in other related plants, such as curculigol A from *C. orchioides* [12] and certain polyunsaturated long chain alkenes having structure similar to β-carotene were also detected. They were not further isolated and characterized because of the very limited amounts obtained.

EXPERIMENTAL

M.p.s: uncorr. ^{1}H NMR (400.13 MHz) and ^{13}C NMR (100.61 MHz): Me_2CO-d_6 or $CDCl_3$ using the solvent peak as int. standard. MS: direct inlet system. UV: MeOH. IR: KBr disc. CD: MeOH.

Plant material

See Ref. [4].

Extraction and isolation

This part of the work is an extension study focused on the isolation of the $CHCl_3$-sol. constituents from the rhizome of *C. capitulata*. The $CHCl_3$ fraction (13 g) from dried powdered rhizomes (5.10 kg) [4] was chromatographed on a silica gel column (70–230 mesh, 500 g), eluted with 0 to 15% MeOH in $CHCl_3$, to give 8 frs. Fr. 1 (36 mg) was recrystallized from $CHCl_3$ to give 2,4-dichloro-5-methoxy-3-methylphenol (**1**, 5.2 mg) as needle crystals. Fr. 2 (85 mg) was rechromatographed on a silica gel column (230–400 mesh, 20 g), eluted with $CHCl_3$ to give vanillin (10 mg) as an amorphous powder. Repeated chromatography on silica gel columns (230–400 mesh) of fr. 4 (396 mg), eluted with 0.5% MeOH in $CHCl_3$, gave amorphous **2** (5.4 mg), **3** (38.2 mg) and 4-hydroxybenzaldehyde (5.0 mg). Fr. 6 (280 mg) yielded ethyl protocatechuate (25.2 mg) after purification on a silica gel column (230–400 mesh, 15 g), eluted with 1.5% MeOH in $CHCl_3$. Fr. 7 (946 mg) yielded 1-*O*-β-D-glucosyl-orcinol (30 mg), 2,6-dimethoxybenzoic acid (16 mg) and 3-*O*-β-D-glucosyl-β-sitosterol (40 mg) *via* repeated silica gel cc (230–400 mesh), eluted with 2 to 10% MeOH in $CHCl_3$.

Peracetylation of **2**

Compound **2** (2.0 mg) was stirred with py-Ac_2O (0.5 ml, 3:1) overnight. Usual work-up yielded the peracetylated product **2a** (2.2 mg).

*2,4-Dichloro-5-methoxy-3-methylphenol (***1***)*

Needle crystals, m.p. 131–132°C. R_f 0.49 (MeOH–$CHCl_3$, 1:19). IR ν_{max} cm^{-1}: 3450, 2940, 1600, 1460, 1420, 1345, 1295, 1230, 1160, 1100, 1080, 945, 820. UV λ_{max} nm (log ε): 229 (*sh*, 3.88), 291 (3.40); MeOH + NaOH: 243 (3.91), 303 (3.65). ^{13}C NMR ($CDCl_3$): δ 154.6 (*s*, C-5), 150.4 (*s*, C-1), 135.1 (*s*, C-3), 114.9 (*s*, C-2), 112.4 (*s*, C-4), 97.8 (*d*, C-6), 56.3 (*q*, OMe), 17.9 (*q*, 3-Me). EIMS *m*/*z* (rel. int.): 210 $[M + 4]^+$ (10), 208 $[M + 2]^+$ (56), 206 $[M]^+$ (100), 191 (12), 163 (30), 99 (20), 69 (25).

*Curlignan (***2***)*

Amorphous powder. R_f 0.25 (MeOH–$CHCl_3$ (1:19). $[\alpha]_D^{23}$ +40.0 (MeOH, *c* 0.5). IR ν_{max} cm^{-1}: 3400, 2940, 1710, 1610, 1520, 1435, 1325, 1205, 1180, 1070, 770; UV λ_{max} nm (log ε): 228 (*sh*, 4.38), 276 (4.19), 298 (*sh*, 3.83); MeOH + NaOH: 231 (*sh*, 4.34), 273 (4.26), 303 (*sh*, 4.10). CD nm ($\Delta\varepsilon$): 317 (0), 293 (+4.82), 280 (+3.96), 271 (+4.88), 245 (+0.07), 240 (+0.73), 229 (−5.69), 211 (+10.27). ^{1}H and ^{13}C NMR (Me_2CO-d_6): Table 1. ^{1}H NMR ($CDCl_3$): δ 7.57 (1H, *br s*, H-4), 7.53 (1H, *br s*, H-6), 6.87 (2H, *br s*, H-2′ and H-6′), 6.86 (1H, *br s*, H-5′), 5.56 (1H, d, J = 7.1 Hz, H-2), 3.90 (1H, *s*, 7-OMe), 3.89 (2H, *m*, H-9a), 3.87 (3H, *s*, 8-OMe), 3.83 (3H, *s*, 3′-OMe), 3.65 (1H, *dt*, J = 7.1, 6.1 Hz, H-3). HR EI MS $[M]^+$ *m*/*z*: 360.1212 ($C_{19}H_{20}O_7$ requires 360.1209); EIMS *m*/*z* (rel. int.): $[M]^+$ 360 (70), 342 (100), 331 (60), 327 (85), 310 (25), 295 (20), 283 (15), 239 (15), 208 (15), 181 (80), 149 (45), 117 (50), 97 (35), 71 (45), 57 (50).

*Curlignan peracetate (***2a***)*

^{1}H NMR ($CDCl_3$): δ 7.56 (1H, *br s*, H-4), 7.55 (1H, *br s*, H-6), 7.00 (1H, *d*, J = 8.0 Hz, H-5′), 6.95 (1H, *d*, J = 1.7 Hz, H-2′), 6.92 (1H, *dd*, J = 8.0, 1.7 Hz, H-6′), 5.58 (1H, *d*, J = 6.8 Hz, H-2), 4.45 (1H, *dd*, J = 11.3, 5.4 Hz, H-9a), 4.30 (1H, *dd*, J = 11.3, 7.5 Hz, H-9b), 3.93 (3H, *s*, 7-OMe), 3.87 (3H, *s*, 8-OMe), 3.80 (1H, *m*, H-3), 3.79 (3H, *s*, 3′-OMe), 2.28 (3H, *s*, 4′-OAc), 2.03 (3H, *s*, 9-OAc). EIMS $[M]^+$ *m*/*z*: 444.

*4-Ethoxy-3-hydroxymethylphenol (***3***)*

Amorphous powder. R_f 0.38 (MeOH–$CHCl_3$, 1:9). IR ν_{max} cm^{-1}: 3300, 2960, 2910, 1500, 1455, 1350, 1200, 1070, 1000, 820, 780 cm^{-1}. UV λ_{max} nm (log ε): 229 (*sh*, 3.76), 297 (3.55); MeOH + NaOH: 253 (*sh*, 3.97), 301 (*sh*, 3.80). ^{1}H NMR ($CDCl_3$): δ 6.71 (1H, *d*, J = 8.6 Hz, H-5), 6.64 (1H, *dd*, J = 8.6, 2.9 Hz, H-6), 6.50 (1H, *d*, J = 2.9 Hz, H-2), 4.60 (2H, *s*, –CH_2OH), 3.57 (2H, *q*, J = 7.0 Hz, OCH_2CH$_3$), 1.24 (3H, *t*, J = 7.0 Hz, OCH$_2$CH_3).

^{13}C NMR ($CDCl_3$): δ 149.8 (*s*, C-4), 148.7 (*s*, C-1), 123.2 (*s*, C-3), 117.1 (*d*, C-6), 115.9 (*d*, C-5), 114.8 (*d*, C-2), 71.8 (*t*, OCH_2CH_3), 66.3 (*t*, $-CH_2OH$), 15.0 (*q*, $-OCH_2CH_3$). EIMS *m*/*z* (rel. int.): $[M]^+$ 168 (30), 122 (100), 94 (70), 66 (15), 55 (10).

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