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(+)-ISOSHINANOLONE AND 2-METHYLBENZOFURAN-4-CARBALDEHYDE FROM THE FISH-STUNNING PLANT *HABROPETALUM DAWEI*

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Key Word Index—*Habropetalum dawei*; Dioncophyllaceae; (+)-isoshinanolone; (+)-*cis*-1,5-dihydroxy-2-methyl-4-oxo-1,2,3,4-tetrahydronaphthalene; 2-methylbenzofuran-4-carbaldehyde.

Abstract—(+)-Isoshinanolone was isolated from an aqueous extract of the leaves of *Habropetalum dawei*. After isolation of (+)-isoshinanolone, the aqueous extract of the leaves was acidified, refluxed and distilled to give a new benzofuran, 2-methylbenzofuran-4-carbaldehyde. (+)-Isoshinanolone was found to have fish-stunning activity.

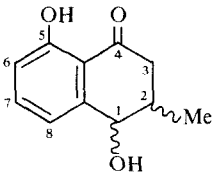
INTRODUCTION

Habropetalum dawei (Hutch. & Dalz.) Airy Shaw belongs to an unusual family, the Dioncophyllaceae. This family consists of three monospecific genera: *Dioncophyllum thollonii*, which is found in Gabon and the Congo Republic; *Triphyophyllum peltatum*, which is found in many areas in Sierra Leone, Liberia and the Ivory Coast; and *H. dawei*, which is found only along a sandy coastal strip that extends for 40–50 miles from Sierra Leone into Liberia. *H. dawei* is used in Sierra Leone to catch fish. The leaves are pounded and thrown into lakes or slow-running streams. Within a few minutes the fish are stunned and float to the surface to be collected by the fishermen.

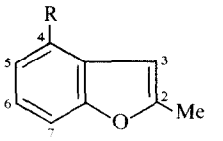
In previous investigations, plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) was isolated from the root bark of *D. thollonii* [1] and plumbagin and naphthalene tetrahydroisoquinoline alkaloids were isolated from *T. peltatum* [2]. The present study was carried out in order to isolate and identify the organic compounds present in an aqueous extract of the leaves of *H. dawei* and to determine their fish-stunning activity.

RESULTS AND DISCUSSION

An oil was isolated from an aqueous extract of the leaves of *H. dawei* by ether extraction and purified by fractional distillation (bp 130° at 0.8 mm Hg, $[\alpha]_D^{18}$ + 33°). Only one compound could be detected in this fraction by TLC and by NMR spectroscopy. The IR spectrum of this compound indicated the presence of OH (3400 cm^{-1}) and of strongly H-bonded C=O (1640 cm^{-1}); C and H, and MS analysis gave a molecular formula of $C_{11}H_{12}O_3$. The compound showed an intense green fluorescence under UV light and its UV spectra in EtOH and M NaOH were similar to those of 2-hydroxyacetophenone. Structure **1** was deduced from the ^1H NMR and ^{13}C NMR spectra. Of importance in the ^1H NMR spectrum is the 2 Hz coupling constant of CHOH (δ 4.65) which is consistent with the OH being 'axial' and *cis* to an 'equatorial' Me. The structure was confirmed by chromic acid oxidation, which gave a yellow crystalline solid (mp 89–91°, $[\alpha]_D^{18}$ −115°), which when dissolved in base and further oxidized with chromic acid gave plumbagin. C and H, and MS analysis of the yellow crystalline solid gave a molecular formula of $C_{11}H_{10}O_3$ and the IR, UV and ^1H NMR spectra were all consistent with its being (−)-5-hydroxy-2-methyl-2,3-dihydro-1,4-naphthoquinone (lit. [3] racemic modification, mp 87°).



1 OH *cis* to Me
1a β-OH, β-Me
1b α-OH, α-Me



2 R = Me
3 R = CHO
4 R = COOH

Structure **1** has been reported for (−)-isoshinanolone isolated from *Diospyros maritima* (Ebenaceae) [4] and the absolute configuration **1a** was tentatively assigned. The MS, ^1H NMR, IR and UV spectra of the compound from *H. dawei* are in good agreement with those of (−)-isoshinanolone, however, (−)-isoshinanolone was obtained as a crystalline solid (decomp. 160°, sublime 230°, melt completely 255°) with $[\alpha]_D^{18}$ −7°. It is not clear how these differences arise, but on the basis of the spectral and optical rotation data, it appears that the compound from *H. dawei* is (+)-isoshinanolone, with the corresponding absolute configuration **1b**.

In order to hydrolyse any glycosides that might be present, the aqueous extract remaining after ether extraction was acidified with hydrochloric acid, refluxed for 6 hr and distilled. Ether extraction of the distillate and fractional distillation of the ether residue gave a pale yellow oil (bp 80° at 0.8 mm Hg). Only one compound could be

detected by TLC and by ^{1}H NMR and ^{13}C NMR spectroscopy; C and H, and MS analysis gave a molecular formula of $C_{10}H_8O_2$. An aldehyde group was indicated by the IR spectrum (1690 cm^{-1}) and by the ^{1}H NMR spectrum (δ 10.10) and this was confirmed by $KMnO_4$ oxidation which gave a carboxylic acid, $C_{10}H_8O_3$. Wolff–Kishner reduction gave 2,4-dimethylbenzofuran (**2**) [5]. Since the physical and spectral properties of the oil and of its carboxylic acid derivative differ from those of known samples of 4-methylbenzofuran-2-carbaldehyde and 4-methylbenzofuran-2-carboxylic acid [5], it follows that the oil is a new benzofuran, 2-methylbenzofuran-4-carbaldehyde (**3**).

(+)-Isoshinanolone was found to have fish-stunning activity when tested by standard procedures [6] using *Barbus liberiensis* as the test fish. In a typical experiment, it was found that (+)-isoshinanolone at a concentration of 25 ppm stunned the test fish in a few minutes and killed them in 45 min. 2-Methylbenzofuran-4-carbaldehyde was found to have bactericidal and fungicidal activity [7].

EXPERIMENTAL

Plant material. H. dawei was collected from the sandy coastal region in the Pujehun District of Sierra Leone; voucher specimens are kept in the Herbarium of the Department of Botany, Fourah Bay College, University of Sierra Leone, Freetown.

Isolation of (+)-isoshinanolone. Chopped, dried leaves (865 g) were extracted at room temp. with H_2O for 2 days and after filtration the extract was extracted with Et_2O. The Et_2O extract was extracted with 5% NaOH and this after acidification with dil HCl was extracted with Et_2O. Evapn of the Et_2O extract, followed by fractional distillation, gave an oil, (+)-isoshinanolone (4 g), bp 130° at 0.8 mm Hg, $[\alpha]_D^{18}$ + 33° ($CHCl_3$; *c* 0.97) (Found: C, 68.7; H, 6.5. $C_{11}H_{12}O_3$ requires: C, 68.7; H, 6.3%); ν_{max}^{film} cm^{-1}: 3400, 2930, 1640, 1453, 1345, 1245, 1163, 978, 813, 797, 742; λ_{max}^{EtOH} nm (log ε): 259 (3.95), 332 (3.56); λ_{max}^{MNaOH} nm (log ε): 260 (3.86), 366 (3.78); ^{1}H NMR (100 MHz, $CDCl_3$): δ 12.3 (1 H, *s*), 7.42 (1 H, *dd*, C-7), 6.87 (2 H, *d*, C-6 and C-8), 4.65 (1 H, *d*, J = 2 Hz, C-1), 2.88 (1 H, *dd*, J = 11 and 17.5 Hz, C-3 *cis* to Me), 2.2–2.6 (2 H, *m*), 1.14 (3 H, *d*, J = 7 Hz, Me); ^{13}C NMR (15 MHz, $CDCl_3$): δ (TMS) 205.1 (*s*, C-4), 162.4 (*s*, C-5), 145.1 (*s*, C-8a), 136.9 (*d*, C-7), 118.9 (*d*, C-6 or C-8), 117.8 (*d*, C-6 or C-8), 114.8 (*s*, C-4a), 70.9 (*d*, C-1), 40.6 (*t*, C-3), 34.4 (*d*, C-2), 16.2 (*q*, Me); MS (probe) *m/z* (rel. int.): 192.0789 (M^+, 100. $C_{11}H_{12}O_3$ requires 192.0786), 177 (M^+ – Me, 24; m* 163.1), 174 (M^+ – H_2O, 18; m* 157.8), 163 (18), 150 (M^+ – 42, 66; m* 117.1), 149 (30), 131 (24), 122 (60), 121 (90).

Oxidation of (+)-isoshinanolone. To a soln of (+)-isoshinanolone (400 mg) in Me_2CO (20 ml) at 0° was added dropwise a CrO_3–H_2SO_4 soln (8 g CrO_3, 7 ml conc H_2SO_4, 12 ml H_2O) until a permanent brown colour was obtained. After 2 min the reaction mixture was poured into ice and H_2O (100 ml) and this was extracted with Et_2O. The extract on evapn gave yellow needles of (–)-5-hydroxy-2-methyl-2,3-dihydro-1,4-naphthoquinone (310 mg), mp 89–91° (from pentane), $[\alpha]_D^{18}$ –115° ($CHCl_3$; *c* 0.53) (Found: C, 69.5; H, 5.4. Calc. for $C_{11}H_{10}O_3$: C, 69.5; H, 5.3%); ν_{max}^{Nujol} cm^{-1}: 1695 (C=O), 1640 (H-bonded C=O); λ_{max}^{EtOH} nm (log ε): 229 (4.37), 261 (3.70), 345 (3.70); ^{1}H NMR (100 MHz, $CDCl_3$): δ 12.1 (1 H, *s*, OH), 7.68 (1 H, *t*, J = 7.5 Hz, C-7), 7.55 (1 H, *dd*, J = 2 and 7.5 Hz, C-6 or C-8), 7.25 (1 H, *dd*, J = 2 and 7.5 Hz, C-6 or C-8), 3.35–3.03 (2 H, *m*, C-2 and C-3 *trans* to Me), 2.83 (1 H, *dd*, J = 12 and 17 Hz, C-3 *cis* to Me), 1.14 (3 H, *d*, J = 6.2 Hz, Me); MS (probe) *m/z* (rel. int.): 190 (M^+, 79), 175 (M^+ – Me, 100), 162 (M^+ – CO, 19), 147 (26), 120 (48), 92 (41). 20 mg was dissolved in M NaOH and an excess of the CrO_3–H_2SO_4 soln was added. After 2 min the reaction mixture was poured into ice and H_2O and this was extracted with $CHCl_3$. The extract on evapn gave orange needles (12 mg) that were identical (IR, UV, ^{1}H NMR, MS, mp, mmp) with a sample of plumbagin.

Isolation of 2-methylbenzofuran-4-carbaldehyde (**3**). The aq. extract (from 865 g of chopped dried leaves), after extraction with Et_2O (see above), was acidified with HCl (4 M, 200 ml), refluxed for 6 hr and distilled. The distillate was extracted with Et_2O and the Et_2O extract was washed with Na_2CO_3 (2 M). Evapn of the Et_2O extract, followed by fractional distillation, gave a pale yellow oil, 2-methylbenzofuran-4-carbaldehyde (2.5 g), bp 80° at 0.8 mm Hg (Found: C, 74.3; H, 5.4. $C_{10}H_8O_2$ requires: C, 75.0; H, 5.0%); ν_{max}^{film} cm^{-1}: 2740 (aldehyde C–H), 1690 (aldehyde C=O), 1590, 1435, 1255, 780; λ_{max}^{EtOH} nm (log ε): 226 (4.24), 276 sh (3.85), 284 (3.92), 325 (3.73); ^{1}H NMR (100 MHz, $CDCl_3$): δ 10.10 (1 H, *s*, CHO), 7.61 (1 H, *d*, J = 7.5 Hz, C-5 or C-7), 7.58 (1 H, *d*, J = 7.5 Hz, C-5 or C-7), 7.30 (1 H, *t*, J = 7.5 Hz, C-6), 7.10 (1 H, *s*, C-3), 2.50 (3 H, *s*, Me); ^{13}C NMR (15 MHz, $CDCl_3$): δ (TMS) 192.5 (*d*, CHO), 159.7 (*s*, C-2 or C-7a), 155.5 (*s*, C-7a or C-2), 128.6 (*s*, C-4), 128.6 (*s*, C-3a), 128.5 (*d*, C-5 or C-6), 123.0 (*d*, C-6 or C-5), 116.5 (*d*, C-7), 103.2 (*d*, C-3), 14.2 (*q*, Me); MS (probe) *m/z* (rel. int.): 160.0524 (M^+, 98. $C_{10}H_8O_2$ requires 160.0524), 159 (100), 131 (59), 103 (15), 77 (28), 51 (20).

Oxidation of **3**. To **3** (200 mg) in H_2O and Me_2CO (1:1, 20 ml) was added $KMnO_4$ (400 mg) and the mixture was left 24 hr at 25° and filtered. The filtrate was made alkaline with NaOH soln (10%), washed with Et_2O and acidified with dil H_2SO_4 to precipitate colourless needles of 2-methylbenzofuran-4-carboxylic acid (**4**) (138 mg), mp 210–212° (from aq. MeOH) (lit. [5] 4-methylbenzofuran-2-carboxylic acid, mp 190°) (Found: C, 67.9; H, 4.6. $C_{10}H_8O_3$ requires: C, 68.2; H, 4.6%); ν_{max}^{Nujol} cm^{-1}: 3200–2500, 1685; λ_{max}^{EtOH} nm (log ε): 218 (4.39), 262 (3.98), 268 (3.98), 302 (3.74); ^{1}H NMR (100 MHz, Me_2CO-d_6): δ 7.92 (1 H, *d*, J = 7.5 Hz, C-5), 7.67 (1 H, *d*, J = 7.5 Hz, C-7), 7.32 (1 H, *t*, J = 7.5 Hz, C-6), 7.05 (1 H, *s*, C-3), 2.52 (3 H, *s*, Me); MS (probe) *m/z* (rel. int.): 176.0473 (M^+, 100. $C_{10}H_8O_3$ requires 176.0473), 175 (23), 159 (32), 131 (94), 130 (32), 103 (17), 77 (50), 51 (58).

Wolff–Kishner reduction of **3**. To **3** (0.74 g) in DIGOL (5 ml) was added KOH (0.66 g) and $N_2H_4 \cdot H_2O$ (0.5 ml) and the mixture was refluxed for 1 hr and then heated to 200° after removing the condenser. After refluxing for a further 3 hr, the mixture was cooled, acidified with HCl (2 M, 10 ml) and extracted with C_6H_6. 2,4-Dimethylbenzofuran (**2**) was isolated (TLC, Si gel) from the C_6H_6 residue as an oil (0.4 g) (Found: C, 82.3; H, 6.7. Calc. for $C_{10}H_{10}O$: C, 82.2; H, 6.9%); ^{1}H NMR and ^{13}C NMR spectra identical with those of a known sample of 2,4-dimethylbenzofuran [5].

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PHENYLBUTANOIDS FROM *ZINGIBER CASSUMUNAR*

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Key Word Index—*Zingiber cassumunar*; Zingiberaceae; structure elucidation and syntheses; 1-arylbut-1,3-dienes; 1-arylbut-1-enes; (*E*)-4-(3′,4′-dimethoxy)but-3-en-1-yl palmitate; aryl aldehydes.

Abstract—Five novel phenylbutanoids have been isolated from the rhizomes of *Zingiber cassumunar*. 3,4-Dimethoxybenzaldehyde and 2,4,5-trimethoxy-benzaldehyde are also reported from the same source.

INTRODUCTION

Recent work from our laboratories has led to the isolation, structure elucidation [1] and syntheses [2] of six novel aromatic compounds. We have further investigated the hexane extract of the rhizomes of the title plant and the details are now reported.

RESULTS AND DISCUSSION

The milled rhizomes of the *Zingiber cassumunar* were extracted exhaustively with hexane in a Soxhlet apparatus. The hexane-soluble fraction [1] was chromatographed on a column of silica gel using hexane–ether as eluant to give four major fractions I–IV. Fraction I was analysed by GC–MS to give two components which were assigned as 4-(3′,4′-dimethoxyphenyl)but-1,3-diene **1** [1] and 4-(3′,4′-dimethoxyphenyl)but-3-ene **2**. Fraction II was further separated by prep. TLC to give a pale yellow oil and a colourless solid. GC–MS analysis of the oil led to the identification of 4-(2′,4′,5′-trimethoxyphenyl)but-1,3-diene **3** [2] and 4-(2′,4′,5′-trimethoxyphenyl)but-3-ene **4**. The colourless solid has been identified as (*E*)-4-(3′,4′-dimethoxyphenyl)but-3-en-1-yl palmitate **5**. Purification of fraction III and IV by prep. TLC yielded 3,4-dimethoxybenzaldehyde **6** and 2,4,5-trimethoxybenzaldehyde **7**, respectively.

The identities of compounds **1**–**5** were further confirmed by comparing their spectral data with those of the synthetic materials [1, 2] (for details, see Experimental). Compounds **6** and **7** were identical with the authentic samples.

The five phenylbutanoids have not been reported before from plant sources. The two benzaldehydes **6** and **7** are known substances, but have not been reported before from this species of *Zingiber*.

EXPERIMENTAL

^{1}H NMR (60 MHz) spectra were recorded in $CDCl_3$ with TMS as an internal standard. Analytical GC was carried out with a stainless steel column (2 m × 3 mm) packed with silicone OV-17 on chromosorb under two different conditions: temp. programmed 120–200°, 5°/min (condition A) and temp. programmed 150–200° at 5° min (condition B). N_2 flow rate at 50 ml/min and FID were employed for both conditions.

Extraction of the milled rhizomes of *Z. cassumunar* and separation of the concd extract into hexane-soluble and less-soluble fractions have been described previously [1]. The hexane-soluble fraction (30.0 g) was chromatographed on a column of silica gel (1.3 kg) using hexane–Et_2O as the eluting solvent to give four major fractions (I–IV, 5.8, 2.4, 1.0 and 8.5 g respectively, all fractions appearing as yellow viscous oil).