SHORT PAPER

J. Chem. Research (S),
2003, 440–441

Clerodendrone, a novel hydroquinone diterpenoid from *Clerodendrum indicum*[1†]

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Chemical investigation of *Clerodendrum indicum* has resulted in the isolation of clerodendrone, a new member of hydroquinone diterpenoids (which are rare in the investigated family, Verbenaceae). The compound was characterised from its spectral (1D and 2D NMR) data and chemical reactions and was found to contain an uncommon structural pattern with beta-methyl dihydrofuran moiety.

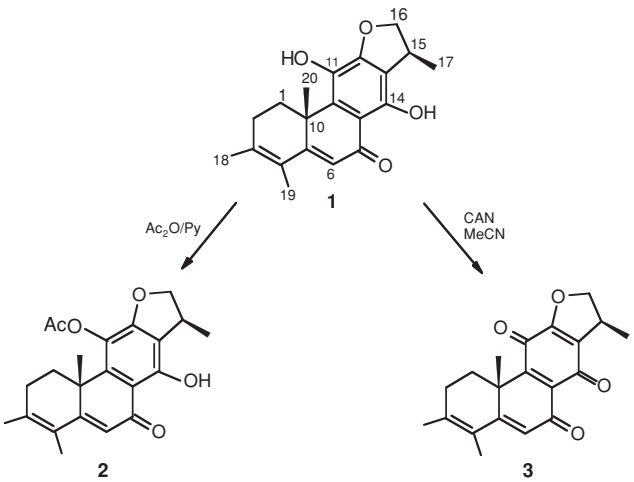
Keywords: *Clerodendrum indicum*, Verbenaceae, stems, hydroquinone diterpenoid, clerodendrone.

Clerodendrum indicum Linn. is a large shrub common in eastern and southern India. The plant is used to treat rheumatism and malaria.[2] Some flavones[3] and cleroindicins[4] were reported earlier from the plant. During our investigation on its bioactive constituents we have recently isolated a new hydroquinone diterpenoid, clerodendrone (**1**) along with two known flavones, hispidulin[3] and 4'-O methyl hispidulin[4] and the known terpenoids, oleanolic acid,[5] oleanolic aldehyde acetate[6] and oleanolic acid acetate[7] from the stem of the plant. Besides hispidulin, the occurrence of all the other compounds in the species has been reported for the first time. Here we describe, structure elucidation and bioactivity of the new hydroquinone, clerodendrone (**1**).

Clerodendrone (**1**) was isolated as orange coloured needles. Its molecular formula was suggested to be $C_{20}H_{22}O_4$ from the mass spectrum (M^+ at *m*/*z* 326) and elemental analysis. The IR spectrum of the compound (υ_{max} 3420, 2925, 1627, 1565, 1457 cm^{-1}) showed the presence of a hydroxyl group chelated with a carbonyl group and an aromatic residue.

The compound was characterised as a hydroquinone diterpenoid having an abietane skeleton from the detailed analysis of its ^1H (1D and 2D) and ^{13}C NMR spectra.

The ^1H NMR spectrum showed the presence of a strongly hydrogen-bonded hydroxyl group (δ13.60, 1H, s) along with another hydroxyl group (δ 4.78, 1H, s), one olefinic proton (δ6.22, 1H, s), two vinylic methyls (δ2.02 and 1.98, 3H each, s) and two other methyls (δ1.38, 3H, d, *J*=7.0 Hz and 1.46, 3H, s). The 2D-HOMCOR spectrum showed that the methyl group at δ1.38 was related to a proton at δ3.80 (m) which was again correlated to two protons at δ4.82, (t, *J*=10.0 Hz) and 4.32, (dd, *J*=10.0, 6.0 Hz) which were attached to the same carbon (C-16). Comparison of the ^1H NMR spectral values of the last two protons with those reported[9] earlier for the protons of a dihydrofuran moiety having a methyl group present in the D-ring of abietane diterpenoids, indicated that the proton resonating downfield (δ4.82) is α-oriented while the other is β. The two protons at C-2 resonated at δ2.48 (1H, m) and 2.22 (1H, dd, *J*=15.5, 7.5 Hz) coupled to each other. Similarly the other two protons at C-1 (δ 3.24, 1H, dd, *J*=15.5, 7.5 Hz and 1.60,1H, m) also showed mutual coupling. A correlation between the protons at C-1 and C-2 was also observed. The downfield shift of one of the protons at C-1 (δ 3.24) is due to the presence of the hydroxyl group at C-11 and the hydroquinone moiety conjugated with a carbonyl group at C-7. A similar effect was also observed[8] earlier with other compounds of similar structure.



The ^{13}C NMR spectrum along with DEPT experiments indicated the presence of twenty carbons including one carbonyl group, two double bonds, one aromatic ring containing three oxygenated carbons, two vinylic methyls and other two methyls, one of which is secondary and the other is tertiary. The assignment of the signals was made by comparing the data with those reported for the other abietane ditepenoids.[8] In the light of these spectral values, the structure of the new diterpene, clerodendrone, was shown to be **1.**

The configuration of the methyl group at C-17 was suggested to be β from the NOESY spectrum of **1**. The methyl protons (δ1.38) showed a correlation with the β-proton (δ4.32) at C-16 while the α-proton (δ 4.82) at this position was correlated to the proton (δ3.80) at C-15.

On acetylation with acetic anhydride and pyridine, clerodendrone (**1**) gave a monoacetate **2.** The ^1H NMR spectrum of the latter clearly showed the signal of an acetyl group (δ2.28, 3H, s). Comparison of the spectrum with that of **1** indicated that one of the protons at C-1 shifted upfield (from δ3.24 to δ2.65) in the acetylated product suggesting that only the hydroxyl group at C-11 of **1** has been acetylated. The chelated hydroxyl group at C-14 could not be acetylated even under reflux.

Treatment of clerodendrone (**1**) with ceric ammonium nitrate (CAN) in MeCN afforded the quinone derivative **3**. The ^1H and ^{13}C NMR spectra of the product (vide Experimenta) clearly supported its structure.

Clerodendrone (**1**) is a new member of hydroquinone diterpenoids which are rare in the family Verbenaceae.[9] The compound is interesting in view of the fact that it has a β-methyl dihydrofuran rather than the α-methyl dihydrofuran

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M).*

found in most of the compounds of the series.[8,9] Clerodendrone (**1**) was found to exhibit antibacterial activity against the gram-positive organisms, *Bacillus subtilis* and *Staphylococcus aureus* (standard : Pencillin G) and gram-negative organisms, *Chromobacterium violaceum,*and *Klebsiella aerogenes* (standard: Streptomycin) in an Agar cup bioassy[11]at a concentration of 30 μg/ml. However, the activity is less than that of the standards.

Experimental

Melting point was measured in a Buchi-510 apparatus. The spectra were recorded with the following instruments: IR, Perkin Elmer; NMR, Varian Gemini-200 MHz; EIMS: VG.Micromass 7070H (70eV). The optical rotation was measured with a JASCO DIP 360 Digital polarimeter. Column chromatography was performed with silica gel (BDH, 100-200 mesh) and TLC with silica gel G.

Plant material: Stems of *C.indicum* L.were collected from West Bengal in March 2000, and identified botanically. A voucher specimen (No CI-St) is preserved in our laboratory and another voucher specimen (IICP-040602) in IICT herbarium.

Extraction and isolation: Air dried stems (3kg) were powdered and extracted with CH_2Cl_2-MeOH (1:1) at room temperature for 120h. The extract was filtered and concentrated by rotary evaporator. The thick brown residue (46g) was chromatographed over silica gel and the column eluted with solvents of increasing polarity using hexane and EtOAC. The fractions eluted were monitored by TLC and the similar fractions were mixed. The following compounds were obtained according to the increasing order of polarity . clerodendrone (60mg), oleanolic aldehyde acetate (30mg), oleanolic acid acetate (150mg), oleanolic acid (10mg), 4'-O-methylhispidulin (60mg) and hispidulin (20mg).

Clerodendrone (**1**): M.p. 220–222^0 C, $[\alpha]^{25}_D$ -29.98^0 (c=0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ13.60 (1H, s, OH - 14), 6.22 (1H, s, H-6), 4.82 (1H, t, *J*=10.0Hz,H-15), 4.32 (1H, dd, *J*=10.0, 6.0Hz, H-15), 3.80 (1H, m, H-16), 3.24 (1H, dd, *J*=15.5, 7.5Hz, H-1), 2.48 (1H, m, H-2), 2.22 (1H, dd, *J*=15.5, 7.5Hz), 2.02 and 1.98 (3H each, s, Me-18, Me-19), 1.60 (1H,m, H-1), 1.46 (3H, s, Me-20), 1.38 (3H, d, *J*=7Hz, Me-17); ^{13}C NMR ($CDCl_3$): δ 190.6 (C-7), 165.5 (C-5), 155.1 (C-14), 154.2 (C-12), 141.1(C-4), 136.2 (C-11), 131.8(C-3), 126.2 (C-13), 119.4 (C-9), 116.2 (C-6), 110.5 (C-8), 81.5 (C-15), 40.4 (C-10), 36.8 (C-16), 30.6(C-2), 29.3 (C-1), 22.2, 20.8 (Me-18, Me-19), 19.6 (Me-20), 15.4 (Me-17); MS. *m/z* (rel.int). 326 (100), 311 (75), 283 (10), 199 (17), 153 (20), 149 (52), 125 (18), 71 (42), 57 (72). Anal.cald.for.$C_{20}H_{22}O_4$. C, 73.60; H,6.79. Found: C, 73.42; H, 6.64.

Acetylation of clerodendrone (**1**): Ac_2O (1ml) and pyridine (5 drops) were added to clerodendrone (5mg). The mixture was stirred overnight. After usual work up the acetylated product **2** was obtained as viscous mass (4mg), ^{1}H NMR ($CDCl_3$): δ 13.8 (1H, s OH-14), 6.22 (1H, s, H-6), 4.80 (1H, t, *J*=12Hz, H-15), 4.25 (1H,dd, *J*=8.0, 7.0Hz, H-15), 3.75 (1H, m, H-16), 2.65-2.20 (3H, m, H_2-2 and H-1), 2.30 (3H, s, OAc), 1.92 and 1.90 (3H each, s, Me-18, Me-19), 1.70 (1H, m, H-1), 1.1.38 (3H, d, *J*=7.0 Hz, Me-17), 1.34 (3H, s, Me-20). MS *m/z* (rel int): 368 (21), 326 (98), 311(47), 167(41), 149 (75), 57(100). Anal. cald for $C_{22}H_{24}O_5$: C,71.70; .H, 6.56. Found; C, 70.81. H, 6.52.

Another mixture of clerodendrone (5mg) in pyridine (1 ml) and Ac_2O (1 ml) was refluxed for 24h but the similar monoacetylated product **2** was obtained

Treatment of clerodendrone with CAN: CAN (50mg) was added to a solution of clerodendrone (25mg) in CH_3CN (10ml). The mixture was stirred for 2h and extract with EtOAc. The mixture was purified by column chromatography to obtain **3** as a solid. ^{1}H NMR ($CDCl_3$): δ6.30 (1H, s, H-6), 4.75 (1H, t, *J*=12.0 Hz, H-15), 4.22 (1H, dd, *J*=10.0, 7.5 Hz, H-15), 3.60 (1H, m, H-16), 2.75-2.20 (3H, m, H_2-2 1and H-1), 1.19 (6H, s, Me-18, Me-19), 1.55 (1H, m, H-1), 1.42 (3H, s, Me-20), 1.36 (3H, d, *J*=7.0 Hz, Me-17)); ^{13}C NMR ($CDCl_3$); δ185.2 (C-14), 184.8 (C-7), 182.6 (C-11), 164.1(C-5), 158.2 (C-9), 154.5 (C-12), 140.5 (C-13), 130.6 (C-4), 125.8 (C-3), 124.6 (C-8), 122.4(C-6), 80.2(C-15), 41.6(C-10), 36.5(C-16), 31.1(C-2), 30.2 (C-1), 24.8, 21.6 (Me-18 and Me-19), 19.2 (Me-20), 14.8 (Me-17).

The authors thank UGC, New Delhi for financial assistance.

Received 23 November 2002; accepted 8 January 2003
Paper 02/1676

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