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Dracophane, a metacyclophane derivative from the resin of *Dracaena cinnabari* Balf.

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Abstract

Dracophane, a novel structural derivative of metacyclophane, was isolated from the resin of *Dracaena cinnabari* Balf. The structure of this compound was determined by spectroscopic methods to be 3,12,21-trihydroxy-1,10,19-tris(4-hydroxyphenyl)-5,14,23-trimethoxy[3.3.3]metacyclophane.

Keywords: *Dracaena cinnabari* Balf.; Agavaceae; Resina; Metacyclophane

1. Introduction

Numerous phenolic compounds belonging to the homoisoflavonoids, flavonoids and chalcones have been isolated from *Dracaena* species (Himmelreich et al., 1995; Masaoud et al., 1995a/–d). Our previous paper (Suchý et al., 1991) described the isolation of homoisoflavans and other constituents from *Dracaena cinnabari* Balf., and determination of the antioxidative activity of some of them. *Dracaena cinnabari* Balf. (Agavaceae) is a tree endemic to the island Socotra (Bellakhdar, 1997). The resin of this tree, dragon's blood, has been used as an astringent in treating diarrhoea and dysentery, as a haemostatic and as an anti-ulcer remedy (Badib, 1991). We now report the isolation and the elucidation of the structure of a further phenolic compound, for which the name dracophane (**1**) is proposed.

2. Results and discussion

The constitution of dracophane (**1**) was determined mainly by NMR spectroscopy. Its planar structure is shown in Fig. 1. ^{1}H and ^{13}C NMR spectra indicated the presence of three structural fragments exhibiting very similar chemical shifts.

Each fragment consists of 1,2,4,5-tetrasubstituted aromatic rings and CH(4–OH–Ph)–CH_2–CH_2 side chains and has been determined as a substituted deoxotetrahydrochalcone. The positions of–OH and–OCH_3 groups were identified by GHMBC (Gradient-selected HMBC) (Wilker et al., 1993) and GSQMBC (Gradient-selected Single-Quantum Multiple Bond Correlation) (Marek et al., 1997) experiments. All the hydrogen atoms of–OH groups form sharp signals at 8.98 ppm with the exception of 3-OH that is shifted to 8.93 ppm. This indicates its unique position in the hydrogen-bonding network. The assignment of protonated carbons was based on the GHSQC (Davis et al., 1992) experiment. The resonances of quaternary carbon atoms were assigned by GHMBC. Furthermore, intraresidual interactions (H-7×C-1, C-8; H-16×C-10, C-17; H-25×C-19, C-26) connecting three deoxotetrahydrochalcone moieties with the 18-membered carbocyclic ring were obtained. The presence of the [3.3.3]metacyclophane skeleton **1** with three exocyclic *p*-phenolic groups was additionally supported by NOE measurement. NOESY experiment (Jeener et al., 1979) indicated the occurrence of dynamic processes in the solution and significant exchange peaks among the signals of H-7, H-16, and H-25 were detected. Chemical environments

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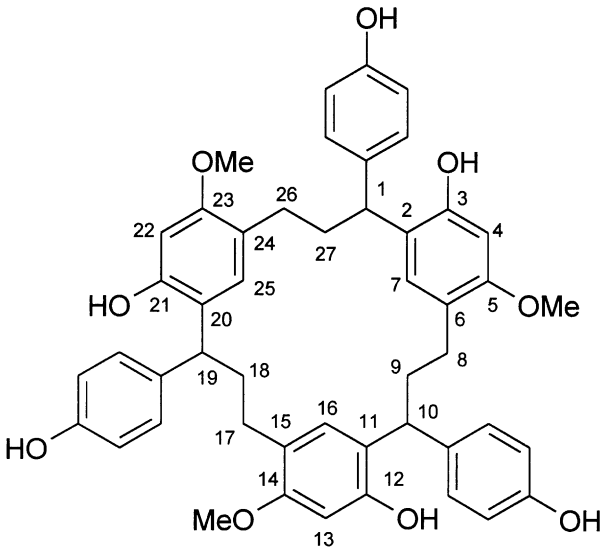
of these hydrogen atoms are probably interchanged due to the conformational changes. These conformational processes, transforming the stereochemical arrangement of one moiety into the other, are sufficiently slow on the NMR time-scale and the separated signals of all the moieties can be observed (Tables 1 and 2). NOE interactions of H-7, H-16, H-25 atoms with meta-protons (H-2′, H-2″, H-2‴) were also detected. Moreover, the interactions H-7 and H-1, H-10; H-16 and H-10; H-19; H-25 and H-19, H-1 indicating the intraresidual as well as the interresidual connectivities were identified. The configurations at the stereogenic centres C-1, C-10, and C-19 have not been determined by NMR spectroscopy due to the chemical exchange process and complex signal pattern. However, the solution of **1** in MeOH was optically active (CD [deg cm^2 dmol^{-1}]: $\theta_{204}+320710.1$, $\theta_{201}-150451.2$). We assume identical relative configurations at the stereogenic carbons and the presence of the only one enantiomer in the sample, 3,12,21-trihydroxy-1,10,19-tris(4-hydroxyphenyl)-5,14, 23-trimethoxy3.3.3 metacyclophane.



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Even under FAB ionization, the molecule underwent electron ionisation because of the highly aromatic nature of this compound, and an M^+ value of 768 was measured. No peaks were observed for $M+H^+$ ions. An accurately measured mass of 768.3307 was obtained at a resolving power of 8000, which corresponds to the elemental composition $C_{48}H_{48}O_9$ (calculated exact mass of 768.3298). In the standard spectrum, the molecular ion eliminates the neutral fragment of phenol along with the adjacent two-carbon chain. This is accompanied by a hydrogen transfer to give an ion peak at $m/z=649$. The accurately measured mass of 649.2843 corresponds to the elemental composition $C_{40}H_{41}O_8$ (calculated exact mass of 649.2801). Linked scans of the product ions in the first field free region at B/E = const. were measured for the $m/z=768$ and $m/z=649$ precursors, respective without using any collision gas. The product ion spectrum of the molecular ion shows peaks at $m/z=648$, 464, 449 (the base peak), 391, and 324. The product ion spectrum of the $m/z=649$ precursor shows peaks at the $m/z=529$, 495, 387, 357, 316, 298 (the base peak), and 266. Both fragmentation series confirm that in addition to the "simple" elimination of the aromatic ring with its adjacentcarbon chain, numerous other rearrangements take place in the fragmentation, which are difficult to interpret.

Synthetic derivatives of [3.3.3.3]metacyclophane and [3.3.3]metacyclophane has been recently prepared and characterized (Burns et al., 2000; Yamato et al., 1998). In addition, longithorols, i.e. metacyclophane-type hydroquinones, have been isolated from the tunicate *Aplidium longithorax* (Fu et al., 1999). To the best of our knowledge this is the first report of a compound with the [3.3.3]metacyclophane skeleton being isolated from a plant.

Although no central atom was found to be complexed with molecule **1**, a cyclic structure containing several phenolic functions can play a significant role in a living organism by coordinating with different molecules including metals. Compound **1** is expected to exhibit biological activity due to the presence of several phenolic groups. The dracophane showed DPPH free radical scavenging activity according to the method (Blois et al., 1958), (unpublished results).

3. Experimental

NMR sample was prepared by dissolving compound **1** (4 mg) in DMSO-d_6 (250 μl) and the solution was inserted into a Shigemi NMR cell. All NMR spectra were recorded on Bruker Avance 300, 500, and 600 spectrometers and chemical shifts are referenced relative to internal TMS. The assignments of NMR signals are based on 2D NOE spectra (mixing time 800 ms) and GHSQC ($^1J_{H,C}=145$ Hz), GSQMBC ($^nJ_{H,C}=7.5$ Hz), and GHMBC ($^nJ_{H,C}=7.5$ Hz) experiments (for experimental details see Marek et al., 1999; Sečkářová et al., 2002). Positive fast atom bombardment mass spectra were recorded on a ZAB-EQ hybrid mass spectrometer (Micromass, Manchester, UK) using xenon at 8 kV as bombarding gas. The compound was dissolved in dimethyl sulfoxide and run in a bis-(2-hydroxyethyl)-disulfide matrix. The analytical HPLC system was HP 1100 equipped with quarternary pump model G1311A and diode array detector model G1315A was used (Agilent Technologies, Palo Alto, California).

3.1. Plant material

Dragon's blood from *Dracaena cinnabari* was collected in Socotra Island of Yemen in autumn 1987. A voucher specimen of resin (RDC02) is deposited at the Department of Natural Drugs, Faculty of Pharmacy, Brno, Czech Republic.

Table 1
^{1}H NMR chemical shifts of dracophane (**1**) in DMSO-d_6 at 303 K

Atom	δ (ppm)	Atom	δ (ppm)	Atom	δ (ppm)
H-1	4.06	H-10	4.13	H-19	4.08
3-OH	8.93	12-OH	8.98	21-OH	8.98
H-4	6.34	H-13	6.36	H-22	6.36
5-OMe	3.63	14-OMe	3.64	23-OMe	3.67
H-7	7.25	H-16	7.08	H-25	7.20
H-8α	2.28	H-17α	2.20	H-26α	2.41
H-8β	2.52	H-17β	2.52	H-26β	2.41
H-9α	2.12	H-18α	2.20	H-27α	2.05
H-9β	2.23	H-18β	2.28	H-27β	2.25
H-2′	7.00	H-2″	6.97	H-2‴	7.01
H-3′	6.58	H-3″	6.56	H-3‴	6.58
4′-OH	8.98	4″-OH	8.98	4‴-OH	8.98

Table 2
^{13}C NMR chemical shifts of dracophane (**1**) in DMSO-d_6 at 303 K

Atom	δ (ppm)	Atom	δ (ppm)	Atom	δ (ppm)
C-1	41.77	C-10	41.19	C-19	41.92
C-2	121.54	C-11	122.43[a]	C-20	122.54[a]
C-3	153.67	C-12	153.59	C-21	153.56
C-4	98.81	C-13	98.91	C-22	99.02
C-5	155.28	C-14	155.41	C-23	154.95
C-6	119.85	C-15	120.15	C-24	120.15
C-7	126.43	C-16	126.88	C-25	127.01
C-8	27.35	C-17	27.90	C-26	25.92
C-9	35.54	C-18	35.11	C-27	36.30
5-OMe	54.87	14-OMe	54.95	23-OMe	55.09
C-1′	136.74[a]	C-1″	136.66[a]	C-1‴	136.66[a]
C-2′	128.22[a]	C-2″	128.28[a]	C-2‴	128.28[a]
C-3′	114.49[a]	C-3″	114.52[a]	C-3‴	114.52[a]
C-4′	154.79[a]	C-4″	154.81[a]	C-4‴	154.81[a]

[a] May be interchanged.

The powdered resin (30 g) was dissolved in EtOH, filtered and evaporated in a vacuum to give a syrupy mass (27 g). A portion of this mass (15 g) was chromatographed on a silica gel (70–230 mesh, Merck) column beginning with a 9:1 mixture of C_6H_6 and Me_2CO and gradually increasing the polarity by increasing the proportion of Me_2CO. A total of 197 fractions (45–50 ml) were collected and pooled according to their TLC behaviour to give 19 fractions. Further purification of the 10th fraction on silica gel column starting with a 9:1 mixture of $CHCl_3$ and MeOH and increasing the polarity by increasing the proportion of MeOH led to the isolation of dracophane, 3,12,21-trihydroxy-1,10,19-tris(4-hydroxyphenyl) - 5,14,23 - trimethoxy[3.3.3]metacyclophane (**1**). Repeated crystallisation with MeOH yielded 20 mg of **1**.

*3.2. 3,12,21-Trihydroxy-1,10,19-tris(4-hydroxyphenyl)-5,14,23-trimethoxy3.3.3.metacyclophane (**1**)*

Pinkish amorphous powder. Mp 293 °C. The purity of (**1**) was verified by HPLC (t_R 17.07 min) on a reverse-phase column (Supelcosil ABZ + Plus 4.6×150 mm, 3 μm). The elution profile had a linear gradient going from 10% MeCN and 90% 40 mM HCO_2H to 100% MeCN in 20 min. The detection of diode array detector at 280 and 330 nm, the flow rate was 1 ml/min, λ_{max}^{MeOH} nm (log ε) 226 (4.69), 286 (4.10). IR $\nu_{max}^{KBr}cm^{-1}$: 3410–3247, 2931, 2959, 1612, 1512. HREIMS *m*/*z* 768.3307 (calculated for $C_{48}H_{48}O_9$, 768.3298). ^{1}H and ^{13}C NMR, see Tables 1 and 2.

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