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The caulindoles: dimeric prenylindoles from *Isolona cauliflora*

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Abstract

Four dimeric prenylindoles occurring in diastereomeric pairs, the caulindole A–D, 5-(3-methyl-2-butenyl)-1*H*-indole, and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole were isolated from the stem and root barks of *Isolona cauliflora*, an ecologically endangered Annonaceae species. Structural determination was achieved based on interpretation of spectroscopic data. Biogenetically, the caulindoles are considered as Diels–Alder-type cycloaddition products of mono- and/or bis-prenylindoles [e.g. 5-(3-methyl-2-butenyl)-1*H*-indole and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole] as the dienes and dienophiles.

Keywords: *Isolona cauliflora*; Annonaceae; Prenylindoles; Caulindoles; Antifungals

1. Introduction

The genus *Isolona* Engl. (Annonaceae) comprises ca. 20 species that are confined to tropical Africa where some of them are used as herbal remedies (Kerharo and Bouquet, 1950; Verdcourt, 1971; Vollesen, 1980). Thus, in the Ivory Coast *Isolona campanulata* Engl. & Diels is used as an aphrodisiac and for increasing fertility in sterile women (Kerharo and Bouquet, 1950). In the Congo *I. hexaloba* Engl. & Diels is used as a purgative and in treating sores, and smoke from the bark as a strained muscle relaxant (Kerharo and Bouquet, 1950).

I. cauliflora Verdc is one of the four *Isolona* species found in Tanzania, growing on an inland island in Sigi valley, in the East Usambara Mountains and bearing flowers at the stem base whereby the fruit-bearing stalks are usually buried in the leaf litter. The plant is quite rare, being reported only in East Africa and thus considered ecologically endangered. Hence it was selected for our on-going analyses of Tanzanian Annonaceae and other plant species for their bioactive and other constituents (Nkunya et al., 1993, 2000; Sung'hwa et al., 1999; Makangara et al., 2002). From the antifungal extracts of the stem and root barks we have isolated four prenylated bis-indole alkaloids, the caulindoles A–D (**1**–**4**), 5-(3-methylbut-2-enyl)-1*H*-indole and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole. In previous investigations, *Isolona* species yielded aporphine alkaloids (Hocquemiller et al., 1978, 1984), and 3,5- and 3,6-(bis)-prenylindoles of the hexalobine type (Achenbach and Löwell, 1995). We now hereby report results from investigations of the root and stem barks of *I. cauliflora*.

2. Results and discussion

Repeated chromatography of the dichloromethane and ethanol extracts of the stem and root barks yielded the caulindoles A–D (**1**–**4**), and the mono-prenylindoles 5-(3-methylbut-2-enyl)-1*H*-indole and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole. Spectroscopic properties of caulindoles A and B were similar to those observed for 5-(3-methylbut-2-enyl)-1*H*-indole and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole, previously isolated from *Monodora* (Annonaceae) and *Riccardia* (liverwort) species, and *Esenbeckia leiocarpa* (Rutaceae) (Adeoye et al., 1986, Benesová et al., 1969; Monache et al., 1989; Nwaji et al., 1972). However, the MS of **1** and **2** exhibited peaks at *m*/*z* 366.2098 ($[M]^+$, $C_{26}H_{26}N_2$) and 366.2107 ($[M]^+$, $C_{26}H_{26}N_2$), and 183.1051 (63%) and 183.1063 (100%), respectively due to mono-prenylindolyl fragment ions corresponding to 5-(3-methylbuta-1,3-dienyl)-1*H*-indole, that would result from *retro*-Diels–Alder fragmentation processes of **1** and **2** respectively, hence suggesting that the caulindoles were dimeric products of

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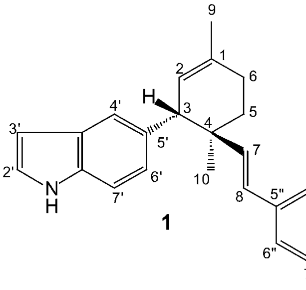

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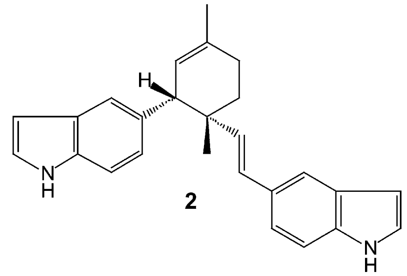

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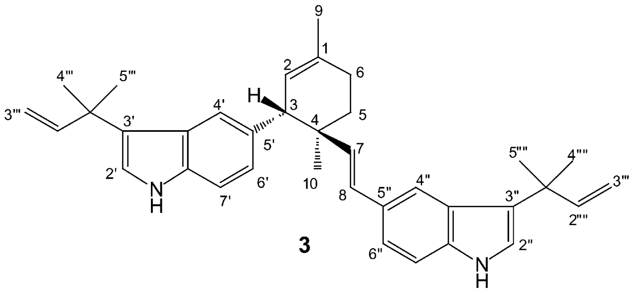

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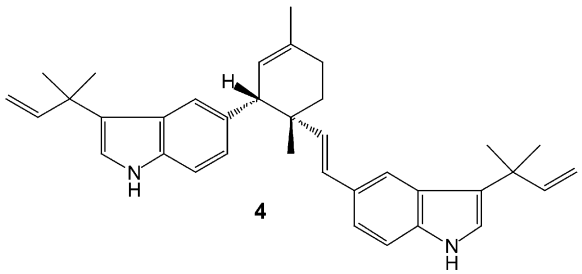

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Diels–Alder-type cycloaddition reactions of prenylindoles such as (E)-5-(3-methylbuta-1,3-dienyl)-1H-indole. Furthermore, both the ^{1}H and ^{13}C NMR spectra of **1** (Table 1) exhibited duplicate signals that correlated with resonances observed for (E)-5-(3-methylbuta-1,3-dienyl)-1H-indole and this suggested presence of structural elements of the latter compound in the molecular framework of **1**. Extensive analysis of 2D NMR correlations for caulindole A [H/H COSY, long-range HETCOR, HMBC, HMQC, and NOE (Table 2)] allowed the assignment of all carbon and proton signals, and structure **1**.

Structure **2** for caulindole B was assigned as indicated for **1** but the positions of the ^{1}H and ^{13}C NMR signals for H-3, H-7 and H-10, C-3, C-7 and C-10 (Table 1) that are close to the chiral carbons C-3 and C-4 were characteristically different and this suggested that the two compounds were diastereomeric. The mono-substitution of both the indolyl moieties in each of the two compounds was evident from the presence of two sets of three indolyl proton signals in the ^{1}H NMR spectrum, indicating a 1,3,4-indolyl substitution relationship (one singlet and two doublets), and one set of signals due to indolyl H-2 and H-3, which hardly coupled with each other. While COSY spectra for the two compounds established homonuclear correlations within each indolyl moiety and HETCOR and HMBC spectra showed long range heteronuclear interactions in **1** (Table 2), specific NOE correlations between protons of the indolyl and the central monoterpene-type units allowed the assignment of the positions of the indolyl moieties, and the relative stereochemical configuration of each compound.

The ^{1}H and ^{13}C NMR spectral data (Table 3) for caulindoles C (**3**) and D (**4**) closely resembled those for the diastereomeric caulindoles A (**1**) and B (**2**), except for the presence of signals due to two 1,1-dimethyl-2-propenyl substituents at indolyl C-3 in both **3** and **4** [absence of H-3 (ca. δ 6.5) and C-3 (ca. δ 102.7) signals of the indolyl units, Table 3] (Achenbach and Renner, 1985; Achenbach and Löwell, 1995). As for compounds **1** and **2**, the MS of each of the caulindoles C and D ($[M]^+$ at m/z 502.3374 and 502.3383 respectively for **3** and **4**, $C_{36}H_{42}N_2$) exhibited the base peaks at m/z 251.1696 and 251.1712, due to fragment ions corresponding to the bis-prenylindolyl units of **3** and **4** respectively, that would be formed through *retro*-Diels–Alder cleavage of each of the two compounds, thus implying a biogenesis of **3** and **4** involving Diels–Alder-type cycloaddition reactions of the corresponding prenylindoles as dienes and dienophiles. Indeed, homonuclear and heteronuclear correlations in the corresponding 2D NMR spectra of **3** and **4** as well as important NOE interactions were in agreement with the proposed structures and the relative stereochemical configurations for caulindole C and D respectively.

Conceivably, the caulindoles would be formed through a Diels–Alder-type cycloaddition reaction involving the mono-prenylindoles such as (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole, with or without enzymatic catalysis. Compound **1** was optically active $\{[\alpha]_D = +13.95^\circ$ $(c = 0.17,\ CHCl_3)\}$, which might have indicated that its biosynthesis was facilitated by the so-called *dielsalderase*-type enzyme (Sanz-Cervera et al., 1993). The conceivable diene and dienophile responsible for the formation of compound **1** [viz. (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole] is unstable and very reactive, and when let to stand under the isolation conditions for a long period, it decomposed without forming any traces of **1** or related compounds, which therefore indicated that caulindole A and hence by analogy, the other caulindoles, were true natural products and not artifacts, as further indicated by the presence of all the caulindoles even in freshly obtained extracts.

Table 1
^1H and ^{13}C NMR spectral data for caulindole A (**1**) and caulindole B (**2**)

H or C	1			2		
	δ_H	*J* (Hz)	δ_C	δ_H	*J* (Hz)	δ_C
1	–	–	133.49	–	–	133.24
2	5.53	*br s*	124.42	5.52	*br s*	125.03
3	3.5	*m*	51.50	3.36	*m*	53.44
4	–	–	38.53	–	–	38.28
5	1.76, 2.11	*m*, *m*	33.44	1.55, 1.85	*m*, *m*	33.65
6	–	–	27.56	2.1, 2.25	*m*, *m*	27.97
7	6.32	*d*, 18	138.39	6.12	*d*, 16.3	135.22
8	6.36	*d*, 18	126.85	6.23	*d*, 16.3	127.05
9	1.81	*s*	23.39	1.81	*s*	23.56
10	0.82	*s*	21.35	1.2	*s*	25.50
1′	8.08	*br s*	–	8.01	*br s*	–
2′	7.17	*br s*	124.38	7.14	*br s*	124.23
3′	6.51	*br m*	102.75	6.45	*br m*	102.75
3a′	–	–	127.44	–	–	127.37
4′	7.44	*s*	121.50	7.45	*s*	121.73
5′	–	–	134.62	–	–	134.62
6′	7.04	*dd*, 8.4, 1.5	124.99	7.02	*dd*, 8.4, 1.5	125.06
7′	7.28	*d*, 8.4	109.61	7.23	*d*, 8.4	109.64
7a′	–	–	135.10	–	–	134.95
1″	8.05	*br s*	–	8.01	*br s*	–
2″	7.17	*br m*	123.95	7.14	*br s*	123.83
3″	6.51	*s*	102.75	6.49	*br m*	102.54
3a″	–	–	128.16	–	–	128.03
4″	7.59	*m*	118.39	7.41	*s*	118.31
5″	–	–	130.56	–	–	130.89
6″	7.29	*m*	120.55	7.07	*dd*, 8.5, 1.5	120.63
7″	7.31	*m*	110.93	7.22	*d*, 8.5	110.72
7a″	–	–	134.21	–	–	134.39

A direct correlation between the NMR signals due to H-10 and H-3, and C-10 and C-3 for compounds **1** and **3,** and **2** and **4** respectively and the relative stereochemical configurations of the caulindoles at the chiral centers was observed and this enabled us to structurally distinguish the diastereomeric caulindoles.

These results demonstrate the ability of Annonaceae species to accumulate monomeric and dimeric prenylindoles (Achenbach and Renner, 1985) that may be phytoalexins, since many of those previously isolated (Achenbach and Löwell, 1995; Nkunya et al., 1996), and the caulindoles A and D had antifungal activities. Thus, caulindole A exhibited mild growth inhibitory activity against the plant pathogenic fungi *Botryodiplodia theobromae*, *Aspergillus niger* and *A. flavus* at 20.5, 18.0 and 18.3% respectively, at a concentration of 2.73 mmol/ml, while caulindole D had growth inhibitory activity against the above fungi at 15.1, 12.5 and 16.0% respectively, at a concentration of 1.99 mmol/ml. 5-(3-Methylbut-2-enyl)-1*H*-indole and (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole showed a similarly mild growth inhibitory activity against the above fungi. The

Table 2
Important HMBC, NOE and HETCOR interactions observed for caulindole A (**1**)

H	HMBC	NOE	HETCOR
2		H-9, H-3, H-4′, H-6′	
3	C-1, C-2	H-2, H-5, H-7, H-8, H-6′	
5	C-1, C-3, C-4, C-6, C-7, C-10	H-3, H-7, H-10	
7	C-3, C-4, C-8, C-10	H-3, H-5, H-10, H-4″	C-5″
8	C-7, C-5″, C-6″	H-3, H-10, H-4″	C-3a″
9	C-1, C-2	H-2	
10	C-3, C-4, C-5, C-7	H-5, H-7, H-8, H-4′, H-6′	C-3, C-5, C-7
3′		H-4′	
4′	C-3	H-3′	
6′	C-3	H-2, H-3, H-10, H-7′	
7′		H-6′	
3″		H-4″	
4″	C-8	H-7, H-8, H-3″	
6″	C-8, C-4″, C-5″		
7″			C-5″

Table 3
^{1}H and ^{13}C NMR spectral data for caulindoles (**3**) and caulindole D (**4**)

H or C	**3**			**4**		
	δ_H	*J* (Hz)	δ_C	δ_H	*J* (Hz)	δ_C
1	–	–	133.80	–	–	133.14
2	5.52	*br s*	125.45	5.52	*br s*	125.00
3	3.34	*m*	52.14	3.49	*m*	53.36
4	–	–	38.52	–	–	38.13
5	1.55, 1.85	*m*, *m*	34.15	1.75	*m*	33.60
6	2.10, 2.20	*m*, *m*	28.15	2.10	*m*	27.8
7	6.10	*d*, 16.3	138.37	6.29	d	134.70
8	6.22	*d*, 16.3	127.43	6.32	d	127.09
9	1.81	*s*	24.01	1.8	*s*	23.35
10	1.25	*m*	21.55	0.8	*m*	25.38
1′	7.8	*br s*	–	7.85	*br s*	–
2′	6.89	*d*, 2.4	120.68	6.93	*d*, 2.4	119.99
3′	–	–	124.13	–	–	123.59
3a′	–	–	125.81	–	–	125.34
4′	7.52	*s*	123.10	7.51	*s*	122.51
5′	–	–	133.44	–	–	132.98
6′	6.99	*dd*, 8.4–1.5	124.58	7.0	*dd*, 8.4, ~1.5	124.17
7′	7.18	*d*, 8.4	110.17	7.23	*d*, 8.4	109.67
7a′	–	–	136.28	–	–	136.15
1″	7.77	*br s*	–	7.81	*br s*	–
2″	6.89	*d*, 2.4	120.26	6.92	*d*, 2.3	119.61
3″	–	–	124.52	–	–	123.95
3a″	–	–	126.53	–	–	125.91
4″	7.41	*s*	120.43	7.56	*s*	119.54
5″	–	–	129.86	–	–	129.68
6″	7.10	*dd*, 8.4, ~1.5	119.76	7.28	*m*	119.44
7″	7.16	*m*	111.56	7.32	*m*	110.78
7a″	–	–	136.80	–	–	135.78
1‴	–	–	37.90	–	–	37.33
2‴	6.13	*dd*, 17.4, 10.5	148.09	6.15	*dd*, 17.4, 10.5	147.67
3‴	5.05, 5.03	*d*, 17.4, *d*, 10.5	110.87	5.07, 5.03	*d*, 17.4, *d*, 10.5	110.20
4‴	1.47	*br s*	28.28	1.45	*br s*	27.85
5‴	–	–	28.28	–	–	27.85
1⁗	–	–	37.90	–	–	37.33
2⁗	6.08	*dd*, 17.4, 10.5	148.09	6.08	*dd*, 17.4, 10.5	147.85
3⁗	5.05, 4.89	*d*, 17.4, *d*, 10.5	111.16	5.05, 4.88	*d*, 17.4, *d*, 10.5	110.42
4⁗	1.44	*s*	28.42	1.42	*s*	27.77
5⁗			28.42			27.95

latter compounds also exhibited a mild activity against the malaria parasite *Plasmodium falciparum* (IC$_{50}$ = 21 μg/ml for each compound).

The fact that prenylindoles occur only in certain genera of the family Annonaceae suggests a chemotaxonomical relationship (Nkunya, 2002), whereby *Annonidium*, *Asteranthe*, *Greenwayodendron*, *Hexalobus*, *Isolona*, *Monodora*, *Polyalthia*, and some *Uvaria* species that accumulate prenylindoles would belong to one sub-family.

3. Experimental

3.1. General experimental procedures

FTIR: CHCl$_3$; ^{1}H NMR at 300 or 400 MHz and ^{13}C NMR at 75 or 100 MHz in CDCl$_3$ or CD$_3$OD, internal standard TMS (^{1}H NMR) and solvent signals (^{13}C NMR); EIMS at 70 eV with direct injection; TLC on plastic plates (Kieselgel 60F$_{254}$, Merck), detection: UV and anisaldehyde/heat (Stahl, 1969). CC: Silica gel 60 (Merck, pet ether/EtOAc, 19:1 v/v), Fractogel® PVA 500 (Merck, CHCl$_3$/MeOH, 1:4 v/v); reversed phase (RP-18, Merck).

3.2. Plant materials

Stem and root barks of *I. cauliflora* Verdc were collected in December 1999 from Namikwe Island, ca. 10 km from Longuza Village, downstream Sigi River in the East Usambara Mountains, Tanga region, Tanzania. Mr. L.B. Mwasumbi of the Herbarium, Botany Department (University of Dar es Salaam) identified the plant species and confirmed the identity at the above herbarium, where a voucher specimen is preserved (Ref. Mwasumbi No. 18713).

3.3. Extraction and isolation

Air-dried pulverized root (RB) and stem barks (SB) were soaked in CH_2Cl_2 and EtOH respectively (2 × 48 h) at room temp. (25–30 °C). Vacuum liquid chromatography (VLC) of the RB extract (pet ether/EtOAc gradient) and then repeated CC [silica gel, pet ether/EtOAc, 19:1 v/v; Fractogel® PVA 500 ($CHCl_3$/MeOH, 1:4 v/v)] gave caulindole A (**1**). CC of the medium polar VLC fraction of the SB yielded 5-(3-methylbuta-2-enyl)-1*H*-indole, (*E*)-5-(3-methylbuta-1,3-dienyl)-1*H*-indole and caulindoles A–D (**1–4**).

3.4. (3,4-*trans*)-3-(5′-Indolyl)-1,4-dimethyl-4-[ethyl-2-(5″-indolyl)enyl]-cyclohex-1-ene (caulindole A) (**1**)

Yellow oil; yield, 11 mg (root bark) and 5.0 mg (stem bark); $[\alpha]_D = +13.95°$ ($c = 0.17$, $CHCl_3$); anisaldehyde: Red; MS, *m/z* (% rel. int.) 366.2098 ($[M]^+$, 6), 183.1051 (63), 168 (100) and 167 (27); IR, υ_{max} 3411, 1471, and 809, 761 cm^{-1}; ^1H and ^{13}C NMR: Table 1.

3.5. (3,4-*cis*)-3-(5′-Indolyl)-1,4-dimethyl-4-[ethyl-2-(5″-indolyl)enyl]-cyclohex-1-ene (caulindole B) (**2**)

Yellow oil; yield, 5.9 mg (stem bark); anisaldehyde: Reddish-yellow; MS, *m/z* (% rel. int.) 366.2107 ($[M]^+$, 12), 183.1063 (100), 168 (93) and 167 (17); IR, υ_{max} 3413 cm^{-1}; ^1H and ^{13}C NMR: Table 1.

3.6. (3,4-*trans*)-3-[3′-(1‴,1‴-Dimethyl-2‴-propenyl)-5′-indolyl]-1,4-dimethyl-4-{2-[3″-(1⁗,1⁗-dimethyl-2⁗-propenyl)-5″-indolyl]-ethyl-2-enyl}-cyclohex-1-ene (caulindole C) (**3**)

Yellow gum; yield, 7 mg (stem bark); anisaldehyde: Purple; MS, *m/z* (% rel. int.) 502.3374 ($[M]^+$, 30), 251.1696 (100), 236 (61), and 222 (12); IR, υ_{max} 3409 cm^{-1}; ^1H and ^{13}C NMR: Table 3.

3.7. (3,4-*cis*)-3-[3′-(1‴,1‴-Dimethyl-2‴-propenyl)-5′-indolyl]-1,4-dimethyl-4-{2-[3″-(1⁗,1⁗-dimethyl-2⁗-propenyl)-5″-indolyl]-ethyl-2-enyl}-cyclohex-1-ene (caulindole D) (**4**)

Yellow gum; yield, 10.7 mg (stem bark); anisaldehyde: Purple. MS, *m/z* (% rel. int.) 502.3383 ($[M]^+$, 11), 251.1712 (100), 236 (92), 222 (29), I82 (19), 168 (18) and 167 (16); IR, υ_{max} 3411 cm^{-1}; ^1H and ^{13}C NMR: Table 3.

3.8. Biological assays

For the antifungal bioassays, mycelia growth measurements were determined in a liquid medium consisting of $NaNO_3$ (0.5 g), K_2HPO_4 (0.5 g), $FeCl_3$ (0.02 g), and dextrose (20 g), each dissolved in sterile water (1000 ml) in a conical flask. The growth medium was dispensed in 18 ml aliquots into 100 ml conical flasks, covered with aluminium foil and then autoclaved at 121 °C for 15 min at 1.2 kg/cm^2. Each of the triplicate test samples (2 ml each) at different concentrations was aseptically pipetted into a flask and a 5 mm mycelia disk of 5-day old fungal isolates was inoculated into each flask. The flasks were incubated at 28 ± 2 °C for 7 days and then the fungal mycelia mats were filtered onto a weighed filter paper, oven-dried at 80 °C for 2 h and the weight of the mycelia determined, whereby the % growth inhibition was calculated as follows:

$$\%\ \text{growth inhibition} = \left(\frac{W_O - W_T}{W_O}\right) \text{x } 100$$

(W_O = weight of mycelia in the control and W_T = weight of mycelia in the treatment). Sterile distilled water (2 ml) was used as the control for each assay.

Antimalarial tests were carried out as previously reported (Nkunya et al., 1991).

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