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The Raputindoles: Novel Cyclopentyl Bisindole Alkaloids from *Raputia simulans*

Konstantina Vougogiannopoulou,[†] Nikolas Fokialakis,[†] Nektarios Aligiannis,[†] Charles Cantrell,[‡] and Alexios-Leandros Skaltsounis*,[†]

Division of Pharmacognosy and Natural Products Chemistry, School of Pharmacy, University of Athens, Panepistimioupoli Zografou, Athens, Greece, and Natural Products Utilization Research Unit, USDA/ARS, National Center for Natural Products Research, University, Mississippi 38677

skaltsounis@pharm.uoa.gr

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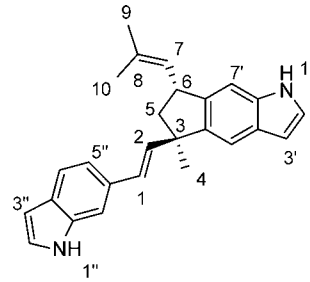
ABSTRACT



Raputindole A

A novel class of bisindole alkaloids is established by the isolation and structural determination of raputindoles A−D (1−4) from the Amazonian plant *Raputia simulans* Kallunki (Rutaceae). Complete spectroscopic characterization was accomplished by means of NMR spectroscopy and APCI (+) HRMS. Raputindoles A−D possess a cyclopentyl moiety fused on the benzene part of the indole ring, originating from the combination of prenylated indole monomers.

Indole alkaloids comprise a large number of diverse metabolites originating from natural sources. The simplicity of the basic indole skeleton has been inspiring pharmaceutical chemists for over a century, and its versatility has made the indole moiety a useful tool in organic chemistry. The indole core is highly reactive; therefore, indole alkaloids possess a variety of biological activities such as antimalarial,[1] antiviral,[2] and antitumor.[3]

In the current study, we report the isolation and structural characterization of raputindoles A−D (**1**−**4**), four novel bisindole alkaloids with a fused cyclopentyl unit deriving from isoprene. Cyclopentyl indole monomers are reported in recent patented works[4] as antiviral agents. Yet, these analogues differ drastically from raputindoles on the origin of the cyclopentyl moiety and, thus, the substitution.

R. simulans was collected from Peru. The stem bark as well as the roots were dried, pulverized, and extracted exhaustively with dichloromethane. The DCM extract of the roots was subjected to medium pressure liquid chromatog-

[†] University of Athens.
[‡] USDA.

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Table 1. NMR Data for Raputindoles A−D in $CDCl_3$

	1		**2**		**3**		**4**	
pos.	δ_C[a]	δ_H[b] (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
1	126.9	6.14 (d, 15.9)	122.2	6.25 (d, 16.0)	131.1	6.29 (d, 16.0)	130.7	6.29 (d, 16.0)
2	137.2	6.45 (d, 15.9)	139.8	6.53 (d, 16.0)	131.2	6.36 (d, 16.0)	131.5	6.36 (d, 16.0)
3	48.4	–	48.7	–	55.1	–	54.9	–
4	27.2	1.61 (br s)	26.8	1.63 (br s)	68.9	4.00 (d, 10.9) a	68.8	4.01 (dd, 10.8, 2.5) a
		–		–		3.90 (d, 10.9) b		3.91 (dd, 10.8, 9.0) b
5	50.0	2.45 (dd, 12.1, 6.8) β	50.0	2.46 (dd, 12.2, 6.8) β	43.9	2.36 (dd, 12.2, 7.0) β	43.6	2.36 (dd, 12.2, 7.1) β
		1.82 (m) α		1.85 (dd, 12.2, 10.6) α		2.06 (dd, 12.2, 10.4) α		2.11 (dd, 12.2, 10.5) α
6	41.0	4.08 (ddd, 10.3, 9.0, 6.8) β	40.5	4.07 (ddd, 10.6, 8.9, 6.8) β	40.6	4.07 (ddd, 10.4, 9.0, 7.0) β	40.8	4.07 (ddd, 10.5, 9.0, 7.1) β
7	128.4	5.22 (d, 9.0)	127.9	5.23 (d, 8.9)	128.3	5.24 (d, 9.0)	128.1	5.24 (dqq, 9.0, 1.1, 0.6)
8	132.6	–	132.2	–	132.7	–	132.8	–
9	25.9	1.82 (br s)	25.9	1.82 (br s)	25.9	1.82 (d, 0.6)	25.8	1.82 (d, 0.6)
10	18.3	1.79 (br s)	18.2	1.78 (br s)	18.3	1.79 (d, 1.1)	18.3	1.79 (d, 1.1)
1′	–	8.04 (br s)	–	8.10 (br s)	–	8.14 (br s)	–	8.08 (br s)
2′	123.7	7.17 (t, 2.7)	124.2	7.20 (t, 2.8)	124.1	7.19 (t, 2.8)	123.9	7.19 (t, 2.8)
3′	102.6	6.53 (br t)	102.5	6.53 (t, 2.8)	102.4	6.51 (t, 2.8)	102.7	6.55 (ddd, 3.3, 2.0, 0.9)
3′a	127.2	–	127.2	–	128.0	–	127.3	–
4′	115.0	7.45 (s)	115.6	7.33 (s)	116.0	7.33 (s)	115.2	7.56 (s)
5′	141.6	–	139.2	–	140.3	–	137.1	–
6′	142.2	–	143.5	–	139.8	–	143.3	–
7′	106.2	7.08 (s)	105.9	7.24 (s)	106.2	7.38 (s)	106.8	7.11 (s)
7′a	135.8	–	135.2	–	135.5	–	136.1	–
1″	–	8.00 (br s)	–	8.17 (br s)	–	8.11 (br s)	–	8.09 (br s)
2″	124.2	7.13 (t, 2.6)	123.9	7.14 (t, 2.8)	123.9	7.15 (t, 2.7)	124.5	7.15 (t, 2.7)
3″	102.7	6.47 (br t)	102.7	6.51 (t, 2.8)	102.9	6.46 (t, 2.7)	102.9	6.47 (ddd, 3.3, 1.8, 0.9)
3″a	127.1	–	128.1	–	128.1	–	128.2	–
4″	120.5	7.50 (d, 8.3)	119.5	7.49 (d, 7.8)	119.0	7.53 (br s)	119.0	7.54 (br s)
5″	118.5	7.15 (dd, 8.3, 1.1)	120.0	7.05 (t, 7.6)	129.3	–	129.5	–
6″	132.3	–	119.8	7.16 (t, 7.3)	120.5	7.23 (dd, 8.4, 1.5)	120.6	7.24 (dd, 8.4, 1.5)
7″	108.9	7.24 (br s)	121.1	–	111.0	7.28 (d, 8.4)	110.9	7.28 (d, 8.4)
7″a	136.3	–	133.1	–	135.4	–	135.3	–

[a] ^{13}C, 150 MHz. [b] ^{1}H, 600 MHz.

raphy on normal phase silica gel, affording an indole-enriched fraction from which **1** and **2** were isolated by means of C_{18} reversed-phase HPLC. The DCM extract of the stem bark was subjected to FCPC fractionation (fast centrifugal partition chromatography, *n*-heptane/AcOEt/MeOH/H_2O 3:1:3:1), resulting in the isolation of **3** and **4** by means of preparative TLC.

Raputindole A (**1**) was isolated as a yellow semisolid and was assigned with the molecular formula $C_{26}H_{27}N_2$, according to APCI (+) HRMS (*m*/*z* 367.2168 $[M + H]^+$, calcd 367.2169). Preliminary examination of 1D NMR data (proton and carbon-13) suggested that **1** is an indole dimer. The latter was apparent not only from the distinct broad peaks of the exchangeable nitrogen protons in the ^1H NMR spectrum but also from the pairs of indole protons (H-2′/H-3′ and H-2″/H-3″) appearing as characteristic pairs of triplet/broad triplet peaks[5] (see Table 1). The ^{13}C NMR data showed a total of 26 carbons, 20 olefinic and 6 aliphatic, and according to the molecular formula, **1** possesses 15 degrees of unsaturation. Therefore, it is evident that the remaining three degrees should be distributed to two double bonds and a closed ring system. HSQC correlations were used to determine the nature of the double bonds as exocyclic: $\Delta^{1,2}$ is in fact trans-bisubstituted, as shown by the double peaks (J = 15.9) corresponding to H-1 and H-2 (δ_H 6.14 and 6.45), whereas $\Delta^{7,8}$ is the double bond of an isobutene group, according to the diagnostic proton signals of H-7 and 3H-9/3H-10 (δ_H 5.22 and 1.82/1.79).

Determination of the substitution pattern of the two indole moieties was based mainly on HMBC correlations. According to ^1H NMR data, H-4″, H-5″, and H-7″ form an ABX spin system (δ_H 7.50 d, 7.15 dd, 7.24 br s). The substituent, in this case the trans double bond, is placed on position 6″, as shown by the HMBC correlations of H-5″ and H-7″ with C-1. On the other hand, the second indole moiety of **1** shows a completely different type of substitution. C-4′ and C-7′ are ortho-substituted, as revealed from the HMBC cross peaks assigned to the correlations of H-4′ with C-6′ (δ_C 142.2) and H-7′ with C-5′ (δ_C 141.6).

HMBC correlations were of vital importance for the determination of the bridging manner of the two indole moieties. The non-indole part of **1** consists of 10 carbon atoms distributed in a trans double bond (C-1, C-2), an isobutene group (C-7 to C-10), the C-4 terminal methyl group (δ_H 1.61, δ_C 27.2), and a five-membered ring system incorporating C-3, C-5, C-6, as well as C-5′ and C-6′ of the indole moiety. The latter was evident from the HMBC correlations of H-4′ with C-3 (δ_C 48.4), H-7′ with C-6 (δ_C 41.0), as well as the strong correlation between H-5β with both C-5′and C-6′. The trans double bond and the C-4 methyl group are both attached to position 3, as shown from the

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HMBC signals of H-1 with the quaternary C-3 and H-4 with C-2, C-3, C-5, and C-5′.

The multiplicity of the peaks corresponding to protons 5, 6, and 7 reflects the observed COSY correlations. Olefinic H-7 of the isobutene group couples with H-6 (δ_H 4.08, ddd, 10.3, 9.0, 6.8), which by turn couples clearly with both H-5. The magnitude of the coupling constant between H-5β and H-6 (J 6.8) reveals that these protons are cis-orientated, whereas the coupling between H-5α and H-6 (J 10.3) is distinctive of trans-orientated protons.

The relative stereochemistry of raputindole A (**1**) was determined according to NOESY data. Strong NOE interactions between H-6 and H-1, H-2 and H-5β along with the correlation observed between H-7 and H-5α indicate that H-6 and the trans double bond are on the same face (see Figure 2). Furthermore, both protons of the trans double bond

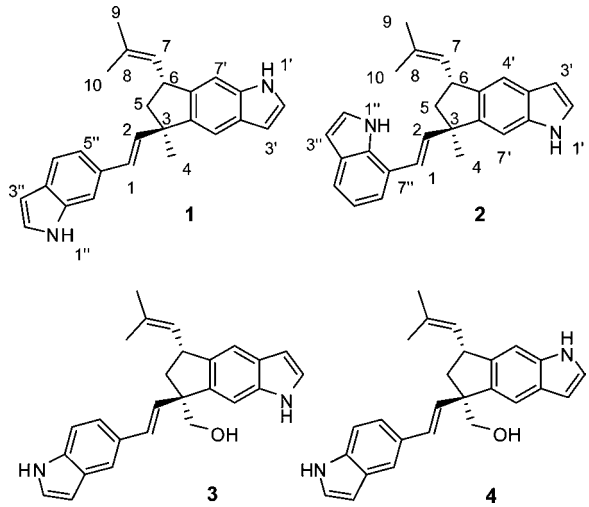

Figure 1. Structural formula of raputindoles A−D (**1**−**4**).

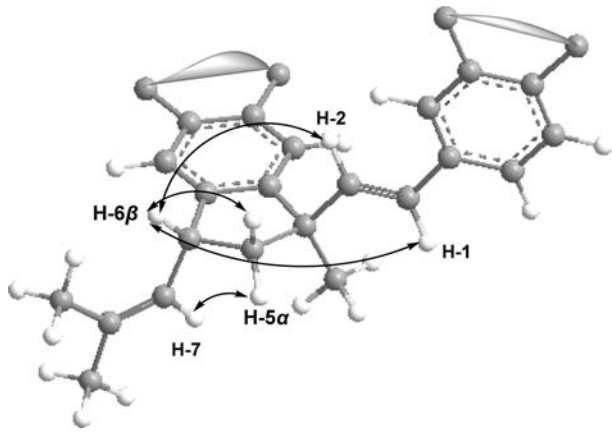

Figure 2. Important NOE interactions observed for raputindoles A−D (**1**−**4**).

interact with H-5β and H-6, in the same manner that H-7 interacts with both H-5α and the indole H-7′. All of the aforementioned suggest that there is free rotation of both double bonds. According to all evidence presented above, raputindole A corresponds to structure **1** (Figure 1).

Raputindole B (**2**) is an isomer of **1**, as shown from the pseudomolecular ion at *m*/*z* 367.2176 in the APCI (+) HRMS. Analysis of 2D NMR data and especially HMBC correlations showed that in this case the trans-bisubstituted double bond is attached to position 7″ of the indole ring. Moreover, the HMBC correlation of H-6 with C-4′ suggests that C-6 of the cyclopentyl moiety is attached to C-5′ of the indole ring. NOESY interactions were exploited for the determination of the relative stereochemistry of **2**. As with raputindole A, we observed strong correlations between H-6 and H-1, and H-2 and H-5β, suggesting that H-6 and the trans double bond are again on the same face. Finally, raputindole B was assigned with structure **2** (Figure 1).

Raputindoles C (**3**) and D (**4**) are isomeric, with pseudomolecular ions at *m*/*z* 383.2133 and 383.2125 (calcd 383.2118 for $C_{26}H_{27}N_2O$), respectively. In comparison with raputindoles A and B, **3** and **4** are larger by 16 Da. Analysis of ^{1}H NMR and ^{13}C NMR data revealed many structural similarities: both compounds possessed an oxygenated carbon atom (C-4), an ABX spin system (H-4″, H-6″, and H-7″ for both compounds) and the same cyclopentyl moiety as raputindoles A and B. For raputindole C (**3**), HMBC correlations revealed that the cyclopentyl C-6 is attached to indole C-5′, whereas for raputindole D (**4**), C-6 is attached to C-6′. Finally, the pattern of relative stereochemistry is consistent between all products, as shown from NOESY experiments. On the basis of the aforementioned results, raputindole C corresponds to structure **3**, whereas raputindole D is assigned to structure **4** (Figure 1).

Raputindoles A−D are optically active ($[\alpha]_D$ +82.8, +25.9, +42.9, and +22.3, respectively), thus implying an enzymatic cyclization of the isoprene units by nucleophilic attack of the benzene indole carbon. The contribution of the isoprene monomers to the final cyclopentyl moiety may be the same in each case, thus two carbon atoms originate from the unit that holds the trans double bond.

Closure of isoprene units into forming a cyclopentyl ring is also reported in flinderoles[6] but only comprising the indole nitrogen and C-2 and C-3 of the indole five-membered ring.

Raputindoles A−D were tested for their potential to inhibit CDK2, GSK-3β, and DYRK1 kinases and were found to have moderate activity (IC_{50} > 10 μM).[7]

Substitution in the benzene indole part accompanied by complete lack of substitution in positions 1, 2, and 3 of the indole ring is quite rare even among synthetic products due to high reactivity of positions 2 and 3 toward electrophilic substitution. In most living organisms, including plants, aromatic substrates may undergo prenylation with the aid of prenyltransferases, leading to an outstanding diversity of

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secondary metabolites.[8] Nevertheless, prenyltransferases capable of C-prenylating the benzene part of an indole moiety have been isolated only from fungi.[9] Nature often utilizes seemingly ordinary building blocks very differently than chemists do, thus resulting in elaborate structures with intriguing chemical properties.

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Supporting Information Available: Experimental procedure, selected NMR (1D, 2D) and HRMS spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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