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A New Class of Spermidine-Derived Alkaloids

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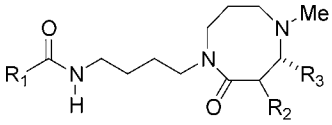
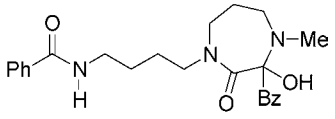
ABSTRACT



1: R_1=R_3=Ph, R_2=H
2: R_1=Me, R_2=H, R_3=Ph
3: R_1=Ph, R_2=Bz, R_3=H



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Four new alkaloids, dovyalicins A–D, were isolated from *Dovyalis macrocalyx*. Their structures were established by two-dimensional COSY, NOESY, HSQC, and HMBC NMR experiments as well as chiroptical methods. The compounds, possessing spermidine as part of a perhydro-1,5-diazocine or a perhydro-1,4-diazepine moiety, constitute a new group of polyamine-derived alkaloids.

Chemical diversity is of the utmost importance in the search for lead compounds with new pharmacological properties. Our ongoing studies on natural products from plants have yielded several compounds with substantial cytotoxic, antiplasmodial, and leishmanicidal activity.[1−5] In this paper, the isolation and structure determination of four novel compounds with hitherto unprecedented skeletons, containing spermidine as part of a perhydro-1,5-diazocine or a perhydro-1,4-diazepine ring system, is reported.

An extract of the leaves of *Dovyalis macrocalyx* (Oliv.) Warb., a plant belonging to a genus of small trees and shrubs found in Africa and Asia (family Flacourtiaceae), was defatted with light petroleum, and the extract was subjected to column chromatography. Alkaloid-containing fractions were further purified by preparative HPLC to give four novel alkaloids **1**−**4**.[6] Compounds **1**[7] and **2**[8] were assigned the molecular formula $C_{24}H_{31}N_3O_2$ and $C_{19}H_{29}N_3O_2$, respectively, as determined by HR-ESI-FTMS. The ^{1}H NMR (400

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(5) Sairafianpour, M.; Kayser, O.; Christensen, J.; Asfa, M.; Witt, M.; Stærk, D.; Jaroszewski, J. W. *J. Nat. Prod.* **2002**, *65*, 1754−1758.

(6) Air-dried and powdered leaves (240 g) were extracted three times with 1 L of CH_2Cl_2/MeOH (1:1). The combined extract was evaporated, and the residue (21.6 g) was partitioned between light petroleum and MeOH/H_2O (9:1). The MeOH/H_2O fraction (7.8 g) was further fractionated on a silica gel column (55 × 3.5 cm i.d.) eluted with CH_2Cl_2 containing 5−50% MeOH and 1% concentrated aqueous ammonia. Alkaloid-containing fractions were identified by TLC using Dragendorff's reagent, and fractions showing similar TLC profiles were pooled to give fractions A−G, which were further purified using isocratic preparative HPLC. A portion of fraction A (160 mg) was chromatographed with CH_2Cl_2/MeOH/DEA (98:1.5:0.5), which gave 68 mg of **1**. Fractions B and C, which contained **1** and some nonalkaloidal impurities as determined by TLC and NMR, were not investigated further. A portion of fraction D (178 mg) was chromatographed with CH_2Cl_2/MeOH/NH_3 (aq) (97:4:0.75), which gave 44 mg of **2**. A portion of fraction F (1 g) was chromatographed with CH_2Cl_2/MeOH/NH_3 (aq) (68:30:2), which gave 60 mg of a mixture containing **3** and **4**, which after repeated preparative HPLC using CH_2Cl_2/MeOH/NH_3 (aq) (96.5:3:0.5) gave 9 mg of **3** and 8 mg of **4**. Plant material used in this work was collected in Kakamega Forest, Kenya (district K5); voucher specimen (DFHJJ36) was deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen, Denmark).

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Table 1. ^{1}H NMR Data of **1**−**4**

	δ_H (multiplicity, J, Hz)[a,b]			
position	**1**	**2**	**3**	**4**
3α	3.16 (dd, 12.9, 11.7)	3.17 (dd, 12.8, 11.7)	4.66 (dd, 10.7, 4.1)	
β	2.53 (dd, 12.9, 3.4)	2.54 (dd, 12.8, 3.4)		
4α			3.20 (dd, 12.8, 10.7)	
β	4.00 (dd, 11.7, 3.4)	4.00 (dd, 11.7, 3.4)	3.10 (dd, 12.8, 4.1)	
5α				2.86 (ddd, 15.1, 12.5, 3.2)
β				3.03 (dm, 15.1)
6α	2.99 (ddd, 15.4, 7.9, 3.0)	3.00 (ddd, 15.4, 7.9, 3.0)	2.48 (ddd, 15.1, 9.6, 3.4)	1.44 (m)
β	2.48 (ddd, 15.4, 7.6, 3.0)	2.50 (ddd, 15.4, 7.6, 3.0)	2.67 (ddd, 15.1, 5.8, 3.9)	2.07 (m)
7α	1.61 (m)	1.63 (m)	1.72 (m)	3.24 (dd, 14.9, 4.8)
β	1.80 (m)	1.80 (m)	1.84 (m)	3.62 (dd, 14.9, 12.5)
8α	3.85 (ddd, 15.2, 12.8, 2.2)	3.85 (ddd, 15.2, 12.8, 2.2)	4.09 (ddd, 15.2, 12.8, 3.0)	
β	3.31 (dt, 15.2, 3.7)	3.31 (dt, 15.2, 3.7)	3.46 (m)	
1′	3.69 (dt, 13.2, 6.3)	3.69 (dt, 13.6, 7.0)	3.78 (dt, 13.4, 7.0)	3.78 (dt, 13.6, 7.0)
	3.21 (m)	3.14 (m)	3.02 (ddd, 13.4, 7.8, 6.0)	3.41 (dt, 13.6, 6.0)
2′	1.69 (m)	1.64 (m)	1.64 (m)	1.75 (m)
3′	1.71 (m)	1.56 (m)	1.52 (m)	1.73 (m)
4′	3.53 (m)	3.30 (m)	3.49 (m)	3.54 (m)
			3.35 (ddd, 13.6, 6.9, 5.5)	
2″	7.27 (*AA*′BB′C)[c]	7.28 (*AA*′BB′C)[c]		
3″	7.33 (AA′*BB*′C)[c]	7.33 (AA′*BB*′C)[c]	7.81 (*AA*′XX′Y)[c]	8.43 (*AA*′MXX′)
4″	7.26 (AA′BB′*C*)[c]	7.26 (AA′BB′*C*)[c]	7.35 (AA′*XX*′Y)[c]	7.44 (AA′M*XX*′)
5″			7.38 (AA′XX′*Y*)[c]	7.56 (AA′*M*XX′)
2‴		1.98 (s)		
3‴	7.86 (*AA*′XX′Y)[c]		7.71 (*AA*′MM′X)[c]	7.81(*AA*′MXX′)
4‴	7.41 (AA′*XX*′Y)[c]		7.36 (AA′*MM*′X)[c]	7.46 (AA′M*XX*′)
5‴	7.47 (AA′XX′*Y*)[c]		7.45 (AA′MM′*X*)[c]	7.48 (AA′*M*XX′)
N−Me	2.25 (s)	2.27 (s)	2.47 (s)	2.41 (s)
NH	7.10 (s, br)	6.55 (s, br)	6.64 (s, br)	6.62 (s, br)
OH				6.13 (s, br)

[a] Data were recorded on a Bruker AMX spectrometer at 400.13 MHz. [b] Chemical shift values are in parts per million using $CDCl_3$ as solvent and TMS as an internal standard. [c] Data were recorded on a Varian Unity Inova spectrometer at 799.74 MHz.

and 800 MHz) and ^{13}C NMR (100 MHz) data (Tables 1 and 2) showed the presence of two monosubstituted benzene rings, one of which showed two downfield-shifted resonances of ortho hydrogens. In addition, analysis of the ^{1}H and the COSY spectrum showed NH−$(CH_2)_4$−N, N−Me, N−$(CH_2)_3$−N, and CH−CH_2 spin systems.

The latter three showed ^{1}H and ^{13}C NMR data comparable with those reported for homaline.[9] This suggested the presence of a hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one moiety, which was confirmed by thorough analysis of NOESY, HSQC, and HMBC spectra. In contrast to the C_2 symmetry observed for homaline, compound **1** showed four different pairs of diastereotopic protons in the NH−$(CH_2)_4$−N (1,4-diaminobutane) spin system. NOEs from the NH hydrogen to the downfield-shifted H-3‴ and correlations in the HMBC spectrum from H-3‴ and H-4′ to C-1‴ showed the presence of the benzamide moiety at N-4′ of the 1,4-diaminobutane portion, whereas correlations from H-1′ to C-2 and C-8 in the HMBC spectrum showed that the nitrogen N-1 is a part of the heterocyclic ring.

Selected NOE correlations observed for **1** are shown in Figure 1. The observed correlations are consistent with the

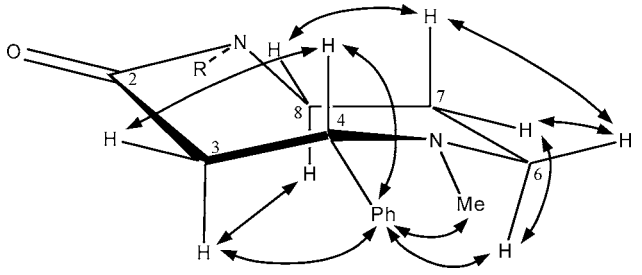


Figure 1. Selected NOEs observed for the hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one moiety of **1**.

conformation of the hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one moiety of homaline as shown by an X-ray study.[10] Optical rotation measurement showed **1** to be levorotatory ($[\alpha]^{25}_D$ −12.4°). The magnitude of the specific rotation is approximately half of that determined for natural homaline[11] ($[\alpha]^{24}_D$ −31°), which has two identical hexahy-

(7) Data for dovyalicin A (**1**): yield 68 mg; colorless gum; $[\alpha]^{25}_D$ −12.4° (*c* 0.45, $CHCl_3$); HR-ESI-FTMS *m*/*z* 394.24909 $[MH]^+$, $[C_{24}H_{32}N_3O_2]^+$ requires 394.24890.

(8) Data for dovyalicin B (**2**): yield 44 mg; colorless gum; $[\alpha]^{25}_D$ −13.7° (*c* 0.68, $CHCl_3$), HR-ESI-FTMS *m*/*z* 332.23321 $[MH]^+$, $[C_{19}H_{30}N_3O_2]^+$ requires 332.23325.

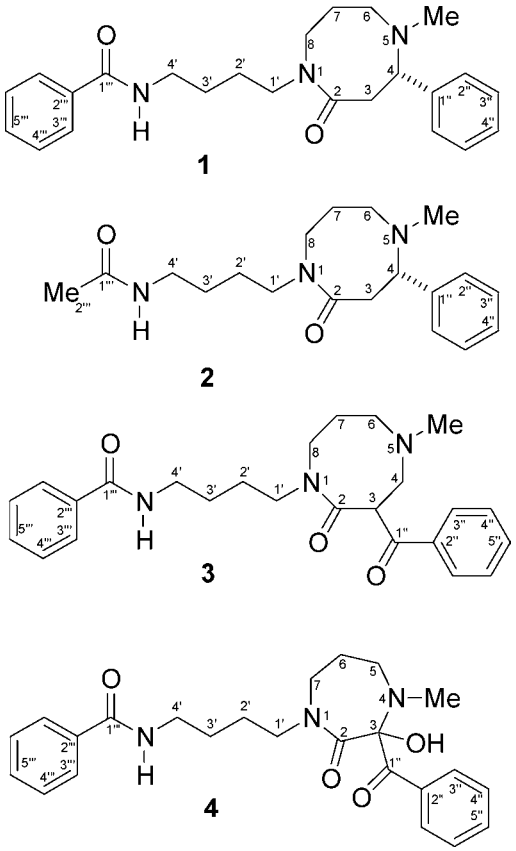
(9) (−)-Homaline is (*S*,*S*)-1,1′-(1,4-butanediyl)bis[hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one]: Crombie, L.; Haigh, D.; Jones, R. C. F.; Mat-Zin, A. R. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2047−2054.

Table 2. ^{13}C NMR Data of **1**−**4**

	δ_C[a,b]			
position	**1**	**2**	**3**	**4**
2	173.7	173.8	172.1	172.9
3	41.0	41.0	52.0	93.6
4	67.9	67.9	59.6	
5				51.8
6	50.9	51.0	54.5	23.4
7	30.1	30.0	30.2	48.9
8	48.5	48.4	47.6	
1′	46.1	46.0	45.2	50.5
2′	25.8	25.7	25.3	25.1
3′	26.2	26.0	26.1	26.2
4′	39.6	39.3	39.2	39.6
1″	141.7	141.6	194.4	195.7
2″	127.5	127.5	136.5	133.8
3″	128.4	128.4	127.8	130.2
4″	127.2	127.2	128.7	128.3
5″			133.1	133.7
1‴	167.6	170.4	167.4	167.7
2‴	134.7	23.3	134.5	134.7
3‴	127.1		127.0	127.0
4‴	128.4		128.5	128.5
5‴	131.2		131.3	131.3
N−Me	43.6	43.7	47.28	33.7

[a] Data were recorded on a Bruker AMX spectrometer at 100.62 MHz. [b] Chemical shift values are in parts per million using $CDCl_3$ as solvent and TMS as an internal standard.

dro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one rings with the (*S*)-configuration at both benzylic centers.[12,13] The CD spectrum of compound **1** showed a negative Cotton effect in the range 210−230 nm ($\Delta\epsilon$ −4.3 L mol^{-1} cm^{-1}, in MeOH), which is also observed for (*S*)-celacinnine, (*S*)-viburnine, and other macrocyclic alkaloids with (*S*)-configuration of stereogenic centers with identical substituents as **1** placed in 13-membered rings.[14] Furthermore, compound **1** showed a negative Cotton effect around 283 nm ($\Delta\epsilon$ $-1.4 \cdot 10^{-2}$ L mol^{-1} cm^{-1}, in MeOH). The band exhibited a vibrational fine structure and is thus attributable to the chiral ^1L_b transition of the benzene chromophore.[15] The negative ^1L_b Cotton effects are also observed for (*S*)-celacinnine and (*S*)-viburnine.[16] Accordingly, compound **1** is formulated as (*S*)-1-(4-benzoylaminobutyl)-hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one.



The ^1H and ^{13}C NMR spectra of **2** were closely related to those of **1**, but the aromatic resonances of the benzoyl group were replaced by those of an acetyl group (δ_C 23.3, δ_H 1.98). Thus, **2** is the acetamide analogue of **1**, which was further supported by full assignment of COSY, NOESY, HSQC, and HMBC spectra. Similar to **1**, compound **2** showed a negative Cotton effect at 278 nm ($\Delta\epsilon$ $-1.9 \cdot 10^{-2}$ L mol^{-1} cm^{-1}, in MeOH) and at 216 nm ($\Delta\epsilon$ −4.7 L mol^{-1} cm^{-1}) and a negative optical rotation ($[\alpha]^{25}_D$ −13.7°).[8] Thus, **2** is (*S*)-1-(4-acetylaminobutyl)-hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one.

Compund **3**[17] was assigned the molecular formula $C_{25}H_{31}N_3O_3$ as determined by HR-ESI-FTMS. The ^{13}C NMR spectrum of **3** showed the presence of an additional carbonyl group (δ 194.4). On the basis of the downfield shift of H-3″ and a HMBC correlation to H-3″, this carbonyl group was attached to C-2″. The appearance of an ABX pattern (δ 4.66, 3.20, and 3.10), resembling that seen for H-4 and H-3 in **1**, might lead to the incorrect conclusion that **3** is the 4-benzoyl analogue of **1**. However, the ^{13}C NMR resonances of C-3 (δ 52.0) and C-4 (δ 59.6) observed with **3** are inconsistent with the structure of the 4-benzoyl analogue of **1**, in which C-4 would be expected to appear around 75 ppm (α effect of benzoyl approximately 7 ppm larger than that of phenyl) and C-3 at 33 ppm (β effect of benzoyl approximately 8

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(16) Although no CD spectra of homaline or similar model compounds are available, application of the quadrant sector rule[15] to the conformation shown in Figure 1, in which the benzene ring plane approximately eclipses H-4, likewise yields the (*S*)-configuration for **1**. Thus, in the (*S*)-configuration (Figure 1), the lower left quadrant and the upper right quadrant are occupied by the Me−N−CH_2−CH_2 ring fragment and the amide group, respectively, both giving negative contributions to the Cotton effect. Note that this Cotton effect is opposite to that observed for (*S*)-α-phenylethylamine: Smith, H. E.; Neergaard, J. R.; Paulis, T.; Chen, F.-M. *J. Am. Chem. Soc.* **1983**, *105*, 1578−1584.
(17) Data for dovyalicin C (**3**): yield 9 mg; colorless gum; $[\alpha]^{25}_D$ −1.1° (*c* 0.79, $CHCl_3$); HR-ESI-FTMS *m/z* 422.24397 $[MH]^+$, $[C_{25}H_{32}N_3O_3]^+$ requires 422.24382.

ppm smaller than that of phenyl). Compound **3** is therefore the 3-benzoyl analogue of **1**. This was proved by connectivities from H-6 and N-Me to C-4 and from H-4 to N-Me and C-3, as well as from H-3 to C-2 and C-4 in the HMBC spectrum. Furthermore, NOEs from H-4 to N-Me and from H-3 to H-3″ confirmed that the second benzoyl is attached to C-3 and not to C-4. Thus, **3** is 3-benzoyl-1-(4-benzoylaminobutyl)-hexahydro-5-methyl-1,5-diazocin-2(1*H*)-one. Selected HMBC connectivities observed for **3** are shown in Figure 2. Compound **3** exhibited a very small specific

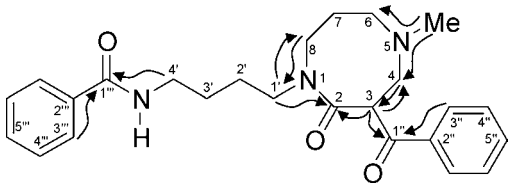

Figure 2. Selected connectivities from HMBC experiment used to establish the structure of **3** (arrows point from H to C).

rotation ($[\alpha]^{25}_D$ −1.1°) and may represent a largely racemic material because the stereogenic center C-3 is part of an enolizable β-dicarbonyl system.

Compound **4**[18] was assigned the molecular formula $C_{24}H_{29}N_3O_4$ on the basis of HR-ESI-FTMS and ^{1}H and ^{13}C NMR data. Combined use of COSY and ^{1}H NMR spectra showed N−$(CH_2)_3$−N, N−Me, and HN−$(CH_2)_4$−N spin systems and two benzoyl groups, together with exchange-broadened signals of NH and OH. Unlike compounds **1**−**3**, compound **4** did not show any ABX pattern in the ^{1}H NMR spectrum. Furthermore, ^{13}C NMR signals around δ 41 and 68, corresponding to C-3 and C-4, respectively, in **1** and **2**, were absent. Instead, a signal of a quaternary carbon at δ 93.6 was observed. This indicated that **4** possesses a perhydro-1,4-diazepine ring oxygenated at C-3 rather than the perhydro-1,5-diazocine ring. This was confirmed by connectivities from H-5 and N−Me hydrogens to C-3 in the HMBC spectrum. Furthermore, NOE correlations from N−Me to OH and from H-3″ to N−Me and H-5 showed that both the OH and the benzoyl group were attached to C-3. Thus, **4** is 3-benzoyl-1-(4-benzoylaminobutyl)-hexahydro-3-hydroxy-4-methyl-1,4-diazepin-2-one. This ring-contracted alkaloid was devoid of optical rotation and is presumably racemic. It may be a product of a nonenzymatic oxygenation of the corresponding β-dicarbonyl precursor.

Although alkaloids derived from spermine have previously been isolated from plants belonging to the family Flacourtiaceae,[10−13,19] spermidine-derived alkaloids with a single perhydro-1,5-diazocine or a single perhydro-1,4-diazepine ring have not been encountered prior to this work.[19] Thus, compounds **1**−**4** are a new class of natural products with a novel carbon skeleton. Pharmacological evaluation of the alkaloids is in progress in our laboratories.

Acknowledgment. Technical assistance of Ms. Jannie Rebø and Ms. Uraiwan Ngamrabiab (The Danish University of Pharmaceutical Sciences) and of Ms. Karen Jørgensen (Department of Chemistry, University of Copenhagen) is gratefully acknowledged. The 800 MHz ^{1}H NMR spectra were obtained using the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules.

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(18) Data for dovyalicin D (**4**): yield 8 mg; colorless gum; $[\alpha]^{25}_D$ 0° (*c* 0.46, $CHCl_3$); HR-ESI-FTMS *m*/*z* 424.22318 $[MH]^+$, $[C_{24}H_{30}N_3O_4]^+$ requires 424.22308.

(19) Bienz, S.; Detterbeck, R.; Ensch, C.; Guggisberg, A.; Häusermann, U.; Meisterhans, C.; Wendt, B.; Werner, C.; Hesse, M. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: San Diego, 2002; Vol. 58, Chapter 2, pp 83−338.