Dovyalicin-Type Spermidine Alkaloids from *Dovyalis* Species

Bonnie Rasmussen,[†] Aimee-Justine Nkurunziza,[†] Matthias Witt,[‡] Hellen A. Oketch-Rabah,[§] Jerzy W. Jaroszewski,[†] and Dan Stærk*,[†]

Department of Medicinal Chemistry, The Danish University of Pharmaceutical Sciences, Universitetsparken 2, DK-2100 Copenhagen, Denmark, Bruker Daltonik GmbH, Fahrenheitstrasse 4, D-28359 Bremen, Germany, and Department of Pharmacology, University of Nairobi, College of Health Sciences, P.O. Box 19676, Nairobi, Kenya

Received May 5, 2006

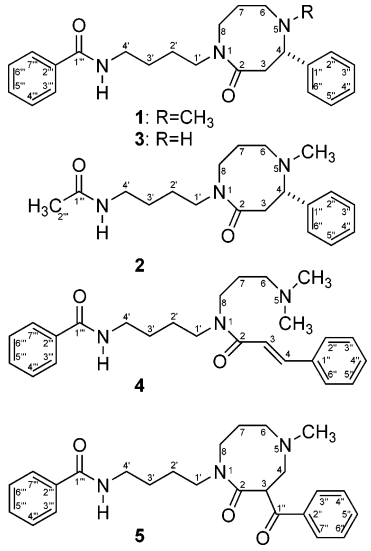
Phytochemical investigations of *Dovyalis abyssinica*, *D. hebecarpa*, and *D. macrocalyx* revealed two new spermidine-type alkaloids, dovyalicin E (**3**) and dovyalicin F (**4**), along with the previously described dovyalicin A (**1**), dovyalicin B (**2**), and dovyalicin C (**5**). In addition, a new phenol glucoside, 4-hydroxytremulacin (**7**), and the new 1,2-cyclohexanediol glucoside **9**, as well as the known compounds methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (**6**) and tremulacin (**8**), were isolated. The structures were established using homo- and heteronuclear two-dimensional NMR experiments and chiroptical methods. At ambient temperature, the *N*-disubstituted amide **4** exists as a mixture of *cis* and *trans* conformers. Variable-temperature ^{1}H NMR studies showed that time-averaged spectra are obtainable at 348 K, and the activation parameters determined for the rotation about the amide bond were $\Delta H^{\ddagger} = 89 \pm 4.6$ kJ/mol, $\Delta S^{\ddagger} = 65 \pm 14$ kJ/mol·K, and $\Delta G^{\ddagger}(298\text{K}) = 70 \pm 4.5$ kJ/mol.

Dovyalis E. Mey. ex Arn. is a small genus comprising 16 species. *Dovyalis* species are frequently found in Central Africa, Mesoamerica, and the northern part of South America. Phytochemical information on the genus is sparse and mostly related to its role as a source of food. Reports include studies on cyclopentenyl fatty acids,[1] tannins,[2] pectin and amino acid composition of *D. caffra*,[3] and ascorbic acid content of *D. hebecarpa*.[4] Pharmacological evaluation of *Dovyalis* species is restricted to a report on antibacterial and antifungal activity of extracts of *D. abyssinica* leaves.[5] However, a recent study demonstrated the presence of a new class of spermidine-type alkaloids in the leaves of *D. macrocalyx*, with dovyalicin A (**1**) as the main alkaloid.[6] In this work, phytochemical investigations of *D. macrocalyx*, *D. abyssinica*, and *D. hebecarpa* are reported. Two new dovyalicin-type alkaloids are described, and the dynamic processes affecting the ^{1}H NMR spectrum of one of these are investigated using variable-temperature NMR. In addition, a new phenol glycoside and a new 1,2-cyclohexanediol glucoside are reported.

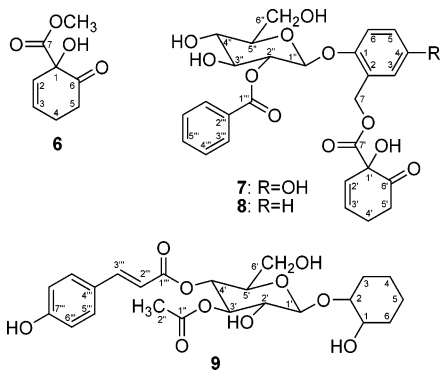
Results and Discussion

Extracts of *D. abyssinica* leaves and twigs, *D. macrocalyx* twigs, and *D. hebecarpa* leaves and twigs were defatted with light petroleum and fractionated on silica gel columns. On the basis of TLC using Dragendorff's reagent for visualization, alkaloid-containing fractions were pooled. From the resulting fractions, alkaloids **1**−**4** as well as compounds **6** and **9** were isolated from *D. abyssinica* leaves by preparative-scale, normal-phase HPLC, and **1**, **4**, and **7** were similarly isolated from *D. abyssinica* twigs. Preparative-scale, normal-phase HPLC afforded compounds **1** and **5** in submilligram quantities from *D. macrocalyx* twigs, and compounds **1**, **7**, and **8** from *D. hebecarpa* twigs and leaves.

Compounds **1** and **2** were identified as dovyalicins A and B, respectively, on the basis of the similarity of ^{1}H and ^{13}C NMR spectroscopic data as well as specific rotation data to those previously reported.[6] The absolute configuration of both compounds is 4*S*, as determined by comparison of their specific rotation with that of natural homaline,[7] for which the absolute configuration was determined by X-ray crystallography and confirmed by synthesis.[8−10]



Compound **3** was assigned the molecular formula $C_{23}H_{29}N_3O_2$ on the basis of HR-ESI-FTMS. ^{1}H and ^{13}C NMR data of **3** (Table 1) were almost identical to those of **1**, except for the absence of a single resonance around δ 43−44 in the ^{13}C NMR spectrum and the absence of the three-proton singlet around δ 2.25 in the ^{1}H NMR spectrum. This indicated that **3** is the N^5-demethyl analogue of **1**, which was confirmed by COSY, NOESY, HSQC, and HMBC experiments (see Supporting Information, Table S1). Compound **3** showed a negative specific rotation ($[\alpha]^{25}_{D}$ −11.7), comparable to



* To whom correspondence should be addressed. Tel: +45 35306413. Fax: +45 35306040. E-mail: ds@dfuni.dk.

[†] The Danish University of Pharmaceutical Sciences.

[‡] Bruker Daltonik GmbH.

[§] University of Nairobi.

10.1021/np060204e CCC: $33.50
Published on Web 08/16/2006

Table 1. ^{1}H (600 MHz) and ^{13}C (100 MHz) NMR Data of **3**

position	chemical shift, $\delta^{a,b}$ ^{13}C	^{1}H
2	173.2	
3	45.3	α: 3.03 (dd, 12.8, 10.9) *β*: 2.52 (dd, 12.8, 2.0)
4	64.7	4.02 (dd, 10.9, 1.9)
5		1.80 (overlap with H-7*β* and H-2′)
6	44.3	α: 3.18 (ddd, 14.8, 12.0, 3.4) *β*: 2.39 (ddd, 14.8, 5.1, 2.4)
7	31.5	α: 1.67 (m) *β*: 1.77 (m)
8	45.4	α: 4.14 (ddd, 15.0, 12.6, 3.9) *β*: 3.27 (ddd, 15.0, 4.7, 1.8)
1′	45.0	3.69 (m) 3.22 (dt, 15.4, 3.4)
2′	25.5	1.71 (m)
3′	26.4	1.65 (m)
4′	39.5	3.51 (m) 3.56 (m)
1″	144.6	
2″/6″	126.8	7.39 (*AA*′BB′C)
3″/5″	128.7	7.32 (AA′*BB*′C)
4″	127.6	7.26 (AA′BB′*C*)
1‴	167.5	
2‴	135.7	
3‴/7‴	127.0	7.84 (*AA*′XX′Y)
4‴/6‴	128.5	7.42 (AA′*XX*′Y)
5‴	131.3	7.48 (AA′XX′*Y*)
NH		6.83 (s, br)

[a] Multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz. [b] Spectra recorded in $CDCl_3$ using TMS as an internal standard.

that observed for dovyalicins A (**1**) and B (**2**), and a negative CD Cotton effect around 216 nm.[6] Thus, the C-4 absolute configuration of **3** is *S*. Compound **3**, (*S*)-1-(4-benzoylaminobutyl)hexahydro-4-phenyl-1,5-diazocin-2(1*H*)-one, is a new alkaloid, for which the name dovyalicin E is proposed.

Compound **4** was assigned the molecular formula $C_{25}H_{33}N_3O_2$ on the basis of HR-ESI-FTMS. The ^{1}H NMR spectrum of **4** acquired at 298 K in DMSO-d_6 showed the presence of two sets of closely spaced resonances in the ratio of 1:1 (Table 2 and Figure 1), whereas the ^{1}H NMR spectrum acquired at 348 K showed only one set of resonances. The high-temperature ^{1}H NMR spectrum showed the presence of a benzoyl group, a spermidine moiety, two isochronous *N*-methyl groups at δ 2.17, and a cinnamoyl moiety with two pairs of doublets of the *trans* double bond (δ 7.08, J = 15.5 Hz, H-3; δ 7.46, J = 15.5 Hz, H-4). A similar pattern of two distinct sets of resonances was observed in the ^{1}H NMR spectra acquired in $CDCl_3$ at 298 K, and a complete analysis of COSY, ROESY, HSQC, and HMBC spectra confirmed the structure **4**. Thus, HMBC correlations were observed from H-4′ and H-3‴ to C-1‴, from H-1′ and H-8 to C-2, and from H-6 to the carbon resonance of the two *N*-methyl groups. Details of correlations found in COSY, ROESY, HSQC, and HMBC spectra are given in the Supporting Information (Table S2). Compound **4**, *N*-(4-benzoylaminobutyl)-*N*-(3-dimethylaminopropyl)-3-phenylpropenamide, is a new compound, for which the name dovyalicin F is suggested.

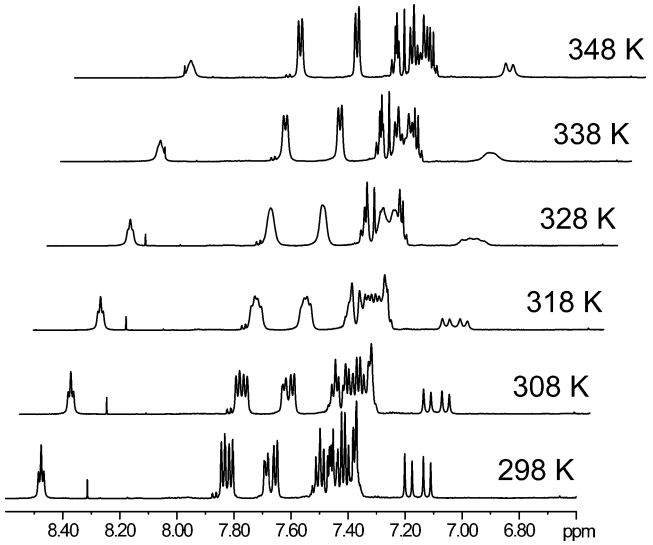


Figure 1. Expansions of 600 MHz ^{1}H NMR spectra of **4** in DMSO-d_6 acquired at temperatures between 298 and 348 K.

The observation of two conformations in room-temperature ^{1}H NMR spectra of compound **4** is not unexpected because of the hindered rotation about the C-2−N-1 amide bond. The energy barrier for interconversion between the *cis* and the *trans* isomer is

Table 2. ^{1}H (600 MHz) and ^{13}C (100 MHz) NMR Data of **4** at Fast and Slow Exchange

position	chemical shift, $\delta^{a,b}$ fast exchange (348 K) ^{1}H	conformation 1 (298 K) ^{13}C	conformation 1 (298 K) ^{1}H	conformation 2 (298 K) ^{13}C	conformation 2 (298 K) ^{1}H
2		165.2		165.0	
3	7.08 (d, 15.5)	118.8	7.19 (d, 15.4)	118.5	7.12 (d, 15.4)
4	7.46 (d, 15.5)	140.8	7.49 (d, 15.4)	141.2	7.49 (d, 15.4)
6	2.28 (t, 6.60)	56.3	2.25 (m)	55.8	2.25 (m)
7	1.68 (q, 7.14)	27.0	1.66 (m)	25.2	1.66 (m)
8	3.45 (m, overlap with H-1′)	44.9	3.49 (m)	44.2	3.49 (m)
1′	3.42 (m, overlap with H-8)	46.9	3.52 (m)	45.4	3.52 (m)
2′	1.60 (m, overlap with H-3′)	26.5	1.59 (m)	24.9	1.59 (m)
3′	1.58 (m, overlap with H-2′)	26.9	1.51 (m)	26.7	1.51 (m)
4′	3.31 (dt, 5.8, 6.7)	38.7	3.29 (m)		3.29 (m)
1″		135.2		135.0	
2″/6″	7.61 (*AA*′XX′Y)	127.8	7.69 (*AA*′XX′Y)	127.7	7.65 (*AA*′XX′Y)
3″/5″	7.38 (AA′*XX*′Y)	128.8	7.39 (m)		7.40 (m)
4″	7.34 (AA′XX′*Y*)	129.3	7.34 (m)		
1‴		166.2		166.0	
2‴		134.7		134.5	
3‴/7‴	7.81 (*AA*′XX′Y)	127.1	7.83 (*AA*′XX′Y)		7.81 (*AA*′XX′Y)
4‴/6‴	7.41 (AA′*XX*′Y)	128.2	7.44 (m)		7.42 (m)
5‴	7.48 (AA′XX′*Y*)	131.0	7.47 (m)		7.43 (m)
N-H	8.19 (s, br)		8.48 (t, 5.6)		
N-Me	2.17 (s)	44.9	2.14 (s)	44.8	2.17 (s)

[a] Multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz. [b] Spectra recorded in DMSO-d_6 using TMS as an internal standard.

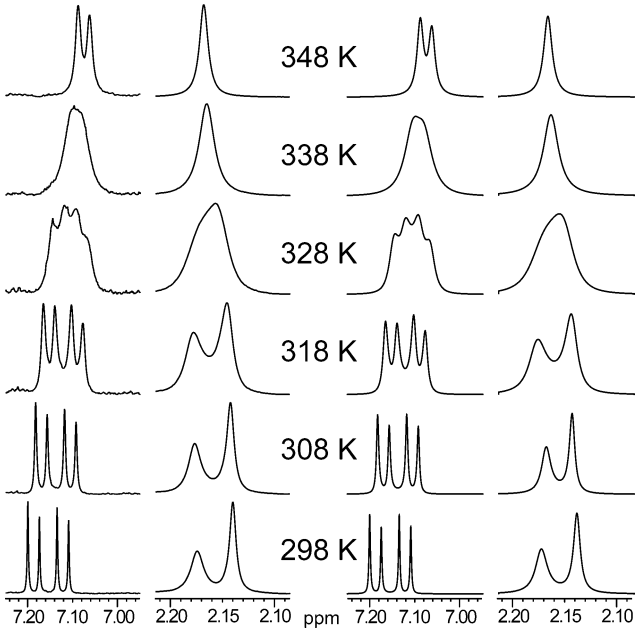


Figure 2. Observed (left) and calculated (right) 600 MHz ^{1}H NMR spectra in DMSO-d_6 of the olefinic proton (H-3) and the *N*-methyl groups of **4**. Rate constants were obtained from the iterative fitting procedure.

generally accepted to be in the range 55−100 kJ/mol,[11−14] which corresponds to slow to intermediate exchange on the NMR time scale at room temperature. For more complex compounds such as acylated polyamines, ^{1}H and ^{13}C NMR spectra acquired under slow exchange conditions are usually not suitable for unambiguous structure elucidation and assignment of resonances.[15] To assess the energy barrier for the *cis*/*trans* amide interconversion in **4** and to obtain ^{1}H NMR reference data under conditions of fast exchange, variable-temperature ^{1}H NMR spectra were acquired. Thus, spectra of **4** dissolved in DMSO-d_6 were recorded at temperatures from 298 to 348 K in steps of 10 K. Expansions of spectral regions between δ 6.6 and 8.6 are shown in Figure 1. The spectrum acquired at 298 K shows separate sets of signals for each conformation in slow exchange. At 348 K, time-averaged signals exhibiting fine structure are observed, which represents the fast exchange regime.

The difference in free energy between the two conformations observed at 298 K is negligible, as shown by equal populations of each conformation observed in the ^{1}H NMR spectrum. The resonances of H-3 and of the *N*-methyl groups were used for full line-shape analysis (Figure 2), which gave exchange rate constants at each temperature. The kinetics of the interconversion were obtained from the Eyring plot, yielding activation parameters $\Delta H^{\ddagger} = 89 \pm 4.6$ kJ/mol, $\Delta S^{\ddagger} = 65 \pm 14$ kJ/mol·K, and $\Delta G^{\ddagger}(298\text{K}) = 70 \pm 4.5$ kJ/mol.

Compound **5** was identified as dovyalicin C on the basis of the similarity of ^{1}H NMR data as well as specific rotation data to those previously reported.[6]

Compound **6** was identified as methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate on the basis of comparison of its ^{1}H and ^{13}C NMR spectra with those reported.[16]

Compounds **7** and **8** were assigned the molecular formulas $C_{27}H_{28}O_{12}$ and $C_{27}H_{28}O_{11}$, respectively, on the basis of the HR-ESI-TOF-MS. ^{1}H and ^{13}C NMR data of **8** (Table 3) were identical to those reported for tremulacin, previously isolated from the bark of *Salix acutifolia*,[17] *Populus tremula*,[18] and *P. tremuloides*[19] (Salicaceae). The structure of **8** was also confirmed by full analysis of COSY, NOESY, HSQC, and HMBC experiments (see Supporting Information, Table S4). The ^{1}H and ^{13}C NMR spectra of **7** resembled those of **8**, except that the characteristic resonances of a 1,2-disubstituted aromatic ring observed in the ^{1}H NMR spectrum of **8** were replaced by the resonances of a 1,2,4-trisubstituted phenyl moiety. The presence of a C-4 hydroxy group in **7** was shown by the upfield shift of H-3 and H-5 (δ 6.61, d, J = 3.0 Hz, H-3; δ 6.67, dd, J = 8.9 and 3.0 Hz, H-5) and by correlations between C-4 and H-3, H-5, and H-6 in the HMBC spectrum. Additional heteronuclear long-range correlations between H2″ and C-1‴, H1″ and C-1, and H-7 and C-7′ proved the linkages between the benzoyl group, the glucosyl residue, the gentisyl alcohol moiety, and the oxocyclohexenecarboxylic acid residue. Details of the structural analysis of **7**, including COSY, NOESY, HSQC, and HMBC spectra, are given in the Supporting Information, Table S3. Compound **7**, 4-hydroxy-2-(1-hydroxy-6-oxocyclohex-2-enecarbonyloxymethyl)phenyl 2-*O*-benzoyl-β-D-glucopyranoside, a hydroxy analogue of tremulacin, is a new compound.

Compound **9** was assigned the molecular formula $C_{23}H_{30}O_{10}$, as determined by HR-ESI-TOF-MS. The ^{1}H NMR spectrum of **9** showed resonances of a *p*-hydroxycinnamoyl group, an acetyl group, and a glucose residue (Table 3). A large downfield shift of H-3′ and H-4′ of the glucose unit (δ 5.21 and 5.01, respectively) and correlations between H-3′ and C-1″ and between H-4′ and C-1‴ in the HMBC spectrum showed the position of the acetyl and the *p*-hydroxycinnamoyl residues. Additional signals from 10 aliphatic hydrogens and six carbon atoms were observed in the ^{1}H and ^{13}C NMR spectra (Table 3). Combined use of COSY, NOESY, HSQC, and HMBC spectra showed that these signals originate from a *trans*-1,2-cyclohexanediol moiety. The attachment of the latter to C-1′ of the glucose residue was confirmed by the NOE correlations between H-1′ and H-1, H-2, and H-3 and by a correlation between H-1′ and C-1 in the HMBC spectrum. Details of the structural analysis of **9**, including COSY, NOESY, HSQC, and HMBC spectra, are given in the Supporting Information, Table S5. Compound **9** is a novel compound. Related structures with *p*-hydroxycinnamoyl or caffeoyl in the 2-position of the glucoside and with a *cis*-1,2-cyclohexanediol moiety have previously been isolated from *Populus grandidentata*,[20,21] *Salix fragilis*,[22] and *S. purpurea*.[23]

The present work demonstrates that the ability to produce dovyalicin-type spermidine alkaloids is a general feature of the genus *Dovyalis*. At the same time, variable-temperature ^{1}H NMR spectroscopy was demonstrated to be a valuable tool for studies of acylated polyamines.[15,24] The genus *Dovyalis* has formerly been classified in the family Flacourtiaceae together with *Homalium*, another genus producing spermine-type alkaloids.[15] Flacourtiaceae has long been recognized as a family having a highly variable and controversial circumscription.[25] Recently, cyanogenic tribes of Flacourtiaceae were separated in the family Achariaceae, and the noncyanogenic tribes, including *Dovyalis* and *Homalium*, were united with Salicaceae.[26] While alkaloids are uncommon in Salicaceae, the phenol glucosides such as tremulacin (**7**) and the closely related salicortin (a debenzoyl derivative of tremulacin) are known to be characteristic markers of Salicaceae. In fact, an isomer of **8**, containing the benzoyl group at O-6 of the glucose moiety rather than O-2 (homaloside B), and similar phenolic glucosides (homalosides A and C) have already been isolated from *Homalium*.[16,27] Thus, the occurrence of phenolic glucosides of this type is in agreement with circumscription of the expanded Salicaceae.

Experimental Section

General Methods. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. NMR spectra were recorded on a Bruker Avance 600 or a Bruker Avance 400 spectrometer (proton frequency 600.13 and 400.13 MHz, respectively) at 25 °C, using TMS as internal standard. NOESY spectra were obtained at 600 MHz with mixing times of 500 or 700 ms. gHMBC and gHSQC spectra were optimized for $^nJ_{C,H}$ = 7.7 Hz and $^1J_{C,H}$ = 145 Hz, respectively. High-resolution mass measurements for exact mass determination were carried out using a Bruker APEX-Q III 7 T ion-cyclotron resonance mass spectrometer equipped with electrospray ionization (ESI) source (Combi source)

Table 3. ^{1}H (600 or 400 MHz) and ^{13}C (100 MHz) NMR Data of **7**, **8**, and **9**

	chemical shift, δ					
	7[a,c,d]		**8**[a,c,e]		**9**[b,c,d]	
position	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	149.6		156		74.0	3.49 (m)
2	127.6		127.5		84.4	3.45 (m)
3	116.5	6.61 (d, 2.9)	129.5	7.14 (dd, 5.5, 1.4)	31.2	ax: 1.31 (m), eq: 2.09 (br d, 11.6)
4	154.2		123.1	6.97 (td, 7.4, 0.9)	24.9	ax: 1.26 (m), eq: 1.71 (m)
5	116.9	6.67 (dd, 8.9, 3.1)	130.1	7.23 (td, 8.3, 1.8)	25.1	ax: 1.26 (m), eq: 1.71 (m)
6	119.5	7.07 (d, 8.8)	115.6	7.02 (d, 7.8)	33.7	ax: 1.27 (m), eq: 1.98 (m)
7	63.9	4.87 (d, 12.5), 4.97 (d, 12.5)	63.6	4.95 (d, 12.6), 5.08 (d, 12.6)		
1′	79.2		78.3		102.4	4.56 (d, 7.8)
2′	129.3	5.68 (dt, 9.8, 1.8)	127.5	5.68 (dt, 10.0, 1.2)	73.0	3.50 (dd, 7.8, 9.6)
3′	133.4	6.13 (dt, 9.8, 3.7)	132.1	6.01 (dt, 10.0, 3.7)	76.5	5.21 (t, 9.6)
4′	27.3	2.50 (m), 2.65 (m)	26.6	2.41 (m), 2.60 (m)	70.3	5.01 (t, 9.6)
5′	36.9	2.49 (m), 2.85 (m)	35.3	2.52 (m), 2.91 (dt, 14.7, 8.0)	75.7	3.67 (m)
6′	207.3		207.5		62.0	3.56 (dd, 12.6, 6.1), 3.66 (m)
7′	171.2		170			
1″	102.3	5.06 (d, 8.0)	99.5	5.15 (d, 7.85)	172.2	
2″	75.7	5.22 (dd, 9.6, 8.0)	74.0	5.30 (dd, 8.6, 8.2)	20.9	1.99 (s)
3″	76.1	3.78 (dd, 9.6, 8.7)	74.8	3.86−3.9 (m)		
4″	71.7	3.54 (dd, 9.7, 8.7)	69.9	3.86−3.9 (m)		
5″	78.4	3.49 (ddd, 9.7, 5.5, 2.3)	76.1	3.51 (m)		
6″	62.6	3.76 (dd, 12.0, 2.3), 3.94 (dd, 12.0, 5.5)	61.3	3.86−3.9 (m)		
1‴	167.3		166.0		167.9	
2‴	131.3		129.5		114.1	6.27 (d, 15.9)
3‴	130.9	8.08 (*AA*′MM′X)	129.9	7.99 (*AA*′MM′X)	147.8	7.62 (d, 15.9)
4‴	129.7	7.48 (AA′*MM*′X)	128.4	7.34 (AA′*MM*′X)	126.9	
5‴	134.5	7.60 (AA′MM′*X*)	133.3	7.49 (AA′MM′*X*)	131.4	7.45 (*AA*′XX′)
6‴					116.9	6.81 (AA′*XX*′)
7‴					161.6	

[a] 600 MHz ^{1}H NMR spectra. [b] 400 MHz ^{1}H NMR spectra. [c] Multiplicity of signals is given in parentheses: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; coupling constants (apparent splittings) are reported as numerical values in Hz. [d] Spectra recorded in CD_3OD using TMS as an internal standard. [e] Spectra recorded in $CDCl_3$ using TMS as an internal standard.

operating in positive-ion mode. The spectra were externally calibrated with collision-induced dissociation spectrum of luteinizing hormone releasing hormone. Samples were dissolved in MeOH and diluted with spray solution, 0.1% HCOOH in MeOH−H_2O (1:1). For compounds **7**−**9**, high-resolution measurements were performed on a Micromass QTOF spectrometer equipped with ESI ion source, operating in positive-ion mode. Spectra were dissolved in MeOH, and poly(ethylene glycol) (PEG) was added for calibration. Column chromatography was performed on Matrex silica gel 60A (particle size 70−200 μm). Alkaloid-containing fractions were monitored using precoated silica gel 60 F_{254} TLC plates with Dragendorff's reagent for visualization. Normal-phase HPLC separations were carried out on a Phenomenex Si60 column (250 × 21.2 mm, particle size 5 μm) eluted with heptane−$CHCl_3$−methanolic NH_3 or $CHCl_3$−methanolic NH_3 mixtures as described below. The separations were performed on a system consisting of two Waters Model 501 pumps, a Waters 484 tunable absorbance detector operated at 254 nm, and a Servogor 120 recorder.

Plant Material. Leaves and stems of *Dovyalis abyssinica* (A. Rich) Warb. and stems of *D. macrocalyx* (Oliv.) Warb. were collected in Kenya (K4, Embu District, Irangi Forrest, and K5, North Kavirondo District, Kakamega Forest, respectively) in April 2001. Leaves and stems of *D. hebecarpa* (Gardner) Warb. were collected in a greenhouse of the Botanical Garden, University of Copenhagen, Copenhagen, Denmark. Voucher specimens of all plants (DFHJJ37, DFHJJ35, and DFHJJ50, respectively) were deposited in Herbarium C (Botanical Museum, University of Copenhagen, Copenhagen, Denmark).

Extraction and Isolation. Powdered leaves (200 g) of *D. abyssinica* were extracted three times with 1 L of CH_2Cl_2−MeOH (1:1). The combined extract was evaporated, and the residue (30 g) was partitioned between MeOH−H_2O (9:1) (1 L) and petroleum ether (1 × 1.5 L and 2 × 1 L). The MeOH−H_2O fraction (27 g) was further fractionated on an open silica column (46 × 3.5 cm i.d.) eluted with CH_2Cl_2 containing 5−50% MeOH and 1% concentrated aqueous NH_3. TLC using Dragendorff's reagent identified alkaloid-containing fractions, and fractions showing similar TLC profiles were pooled into seven fractions, A−G, which were further purified by normal-phase HPLC. Fraction B was chromatographed with $CHCl_3$−saturated methanolic NH_3 (99:1), which gave 13.8 mg of **1**. Fraction D was chromatographed with $CHCl_3$−saturated methanolic NH_3 (99:1), which gave 14 mg of **9**. Fraction E was chromatographed with $CHCl_3$−saturated methanolic NH_3 (99:1) to give 87 mg of a mixture of **1** and **3**, which after repeated purification using the same solvent gave 7.5 mg of **1** and 8.6 mg of **3**. Fraction F was further fractionated on an open silica column (71 × 2 cm i.d.) eluted with $CHCl_3$−MeOH−concentrated aqueous NH_3 (92:7:1), which gave fractions F1−F3 (31, 22, and 35 mg, respectively). Fraction F1 was chromatographed with $CHCl_3$−saturated methanolic NH_3 (99.5:0.5) to give 4.6 mg of **3** and fraction F1.2, which was further chromatographed with heptane−$CHCl_3$−concentrated aqueous NH_3 to give 4.7 mg of **6**. Fraction F2 contained **2** but was not further purified. Fraction F3 was chromatographed twice with $CHCl_3$−saturated methanolic NH_3 (99.5:0.5 and 99:1, respectively), which gave 6.4 mg of **2**. Fraction G was chromatographed twice with $CHCl_3$−MeOH−saturated methanolic NH_3 (95:4:1 and 97:2:1, respectively), which gave 1.6 mg of **4**.

Powdered twigs (532 g) of *D. abyssinica* were extracted four times with CH_2Cl_2−MeOH (1:1) (1 × 3 L and 3 × 2 L). The combined extract was evaporated, and the residue (35 g) was partitioned between MeOH−H_2O (9:1) (1 L) and petroleum ether (3 × 1 L). The MeOH−H_2O fraction (30 g) was further fractionated on an open silica column (50 × 3.5 cm i.d.), eluted with CH_2Cl_2 containing 5−50% MeOH and 1% concentrated aqueous NH_3. TLC using Dragendorff's reagent identified alkaloid-containing fractions, and fractions showing similar TLC profiles were pooled into three fractions, A−C, which were further chromatographed by normal-phase HPLC. White crystals precipitated from fraction B, which after filtering and washing afforded 995 mg of **7**. The mother liquor of fraction B was further fractionated on an open silica column (79 × 2 cm i.d.) eluted with $CHCl_3$ containing 5−10% MeOH and 1% concentrated aqueous NH_3, which gave fractions B1−B4. Fraction B1 (26.7 mg) was purified by HPLC with $CHCl_3$−saturated methanolic NH_3 (99:1), which gave 5.1 mg of **1**. Fraction C was further fractionated on an open silica column (71 × 2 cm i.d.) eluted with $CHCl_3$−MeOH−aqueous NH_3 (9:1:0.1), which gave fraction C1−C3. Fraction C2 (38.4 mg) was purified by HPLC with $CHCl_3$−MeOH−concentrated aqueous NH_3 (94:5:1) to give 9.9 mg of **4**.

Powdered twigs (250 g) of *D. macrocalyx* were extracted with CH_2Cl_2−MeOH (1:1) (1 × 1.5 L and 2 × 1 L). The combined extract was evaporated, and the residue (5.9 g) was partitioned between MeOH−H_2O (9:1) (1 L) and petroleum ether (6 × 500 mL). The MeOH−H_2O fraction (4.1 g) was further fractionated on an open silica column (73 × 3.5 cm i.d.) eluted with CH_2Cl_2 containing 10−40% MeOH and 1% concentrated aqueous NH_3. Alkaloid-containing fractions, as determined by TLC using Dragendorff's reagent, were pooled into two fractions, A and B, which were further fractionated by normal-phase HPLC. Fraction A was chromatographed with CH_2Cl_2−MeOH−diethylamine (98:1.5:0.5) and then with CH_2Cl_2−MeOH−concentrated aqueous NH_3 (97:2.75:0.25), to give 0.3 mg of **1**. Fraction B was chromatographed with CH_2Cl_2−MeOH−concentrated aqueous NH_3 (9:1:0.1), which after repeated purification with CH_2Cl_2−MeOH−concentrated aqueous NH_3 (98.75:1.25:0.1) gave 0.4 mg of **5**.

Powdered twigs and leaves (100 g) of *D. hebecarpa* were extracted with CH_2Cl_2−MeOH (1:1) (2 × 0.5 L and 1 × 1 L). The combined extract was evaporated, and the residue (26 g) was partitioned between MeOH−H_2O (9:1) (0.5 L) and petroleum ether (9 × 250 mL). The MeOH−H_2O fraction (21 g) was further fractionated on an open silica column (37 × 10 cm i.d.), eluted with CH_2Cl_2 containing 10−50% MeOH and 1% concentrated NH_3. TLC using Dragendorff's reagent identified alkaloid-containing fractions, and fractions showing similar TLC profiles were pooled into five fractions, A−E, which were further fractionated by normal-phase HPLC. Fraction A was chromatographed twice with CH_2Cl_2−MeOH−concentrated aqueous NH_3 (97:2.75:0.25), which gave 21.7 mg of **1**. Fraction D was chromatographed with CH_2Cl_2−MeOH−concentrated aqueous NH_3 (8.7:1.2:0.1), which gave 284 mg of **8**. Fraction E contained large amounts of white precipitate, which after filtering and washing afforded 3.6 g of **7**.

Dovyalicin A [(*S*)-1-(4-benzoylaminobutyl)hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one] (1): colorless gum; $[\alpha]^{25}_D$ −10.4 (*c* 0.51, $CHCl_3$), lit.[6] −12.4; ^1H and ^{13}C NMR spectra as previously reported.[6]

Dovyalicin B [(*S*)-1-(4-acetylaminobutyl)hexahydro-5-methyl-4-phenyl-1,5-diazocin-2(1*H*)-one] (2): colorless gum; $[\alpha]^{25}_D$ −23.5 (*c* 0.39, $CHCl_3$), lit.[6] −13.7; ^1H and ^{13}C NMR spectra as previously reported.[6]

Dovyalicin C [1-(4-benzoylaminobutyl)hexahydro-5-methyl-3-benzoyl-1,5-diazocin-2(1*H*)-one] (5): colorless gum; $[\alpha]^{25}_D$ −1.1 (*c* 0.79, $CHCl_3$), lit.[6] −1.1; ^1H and ^{13}C NMR spectra as previously reported.[6]

Dovyalicin E [(*S*)-1-(4-benzoylaminobutyl)hexahydro-4-phenyl-1,5-diazocin-2(1*H*)-one] (3): colorless gum; $[\alpha]^{25}_D$ −11.7 (*c* 0.42, $CHCl_3$); ^1H and ^{13}C NMR data in Table 1; HR-ESI-FTMS *m*/*z* 380.23296 $[MH]^+$, $C_{23}H_{30}N_3O_2$ requires 380.23325, *m*/*z* 402.21501 $[M + Na]^+$, $C_{23}H_{29}N_3O_2Na$ requires 402.21520.

Dovyalicin F [*N*-(4-benzoylaminobutyl)-*N*-(3-dimethylaminopropyl)-3-phenylpropenamide] (4): colorless gum; ^1H and ^{13}C NMR data in Table 2; HR-ESI-FTMS *m*/*z* 408.26428 $[MH]^+$, $C_{25}H_{34}N_3O_2$ requires 408.26455.

Methyl 1-hydroxy-6-oxocyclohex-2-enecarboxylate (6): colorless gum; $[\alpha]^{25}_D$ −185.9 (*c* 0.59, $CHCl_3$); ^1H NMR ($CDCl_3$, 600 MHz) δ 6.13 (1H, dt, J = 9.8, 3.6 Hz, H-3), 5.79 (1H, ddd, J = 9.8, 2.2, 1.7 Hz, H-2), 4.19 (1H, br s, OH), 3.80 (3H, s, OCH_3), 3.00 (1H, dt, J = 14.5, 8.1 Hz, H-5A), 2.78 (1H, m, H-4A), 2.68 (1H, ddd, J = 14.5, 6.3, 3.6 Hz, H-5B), 2.58 (1H, m, H-4B); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 205.4 (C-6), 170.4 (C-7), 131.9 (C-3), 127.7 (C-2), 78.0 (C-1), 53.5 (OCH_3), 35.1 (C-5), 26.9 (C-4).

4-Hydroxytremulacin [4-hydroxy-2-(1-hydroxy-6-oxocyclohex-2-enecarbonyloxymethyl)phenyl 2-*O*-benzoyl-*β*-D-glucopyranoside] (7): yield 995 mg (*D. abyssinica* twigs) and 3.6 g (*D. hebecarpa* twigs and leaves) as white precipitate; $[\alpha]^{25}_D$ −97.4 (*c* 0.63, CH_3OH); ^1H and ^{13}C NMR data in Table 3; HR-ESI-TOF-MS *m*/*z* 567.1479 $[M + Na]^+$, $C_{27}H_{28}O_{12}Na$ requires 567.1473.

Tremulacin [2-(1-hydroxy-6-oxocyclohex-2-enecarbonyloxymethyl)phenyl 2-*O*-benzoyl-*β*-D-glucopyranoside] (8): yield 284 mg (*D. hebecarpa* twigs and leaves) as white precipitate; $[\alpha]^{25}_D$ −134.7 (*c* 0.59, CH_3OH), lit.[19] −126.5 (*c* 2.53, CH_3OH); ^1H and ^{13}C NMR data in Table 3; HR-ESI-TOF-MS *m*/*z* 551.1530 $[M + Na]^+$, $C_{27}H_{28}O_{11}Na$ requires 551.1524.

***trans*-2-{3-*O*-Acetyl-4-*O*-[(*E*)-4-hydroxycinnamoyl]-*β*-D-glucopyranosyloxy}cyclohexanol (9):** yield 14 mg (*D. abyssinica* leaves) as white crystals; $[\alpha]^{25}_D$ −40 (*c* 0.72, CH_3OH); ^1H and ^{13}C NMR data in Table 3; HR-ESI-TOF-MS *m*/*z* 489.1749 $[M + Na]^+$, $C_{27}H_{28}O_{11}Na$ requires 489.1731.

Dynamic NMR Experiments. Variable-temperature ^1H NMR measurements with dovyalicin F (**4**) were performed with a sample dissolved in DMSO-d_6 and thermostated by means of a stream of liquid nitrogen vapor heated to required temperatures using a temperature controller. Temperature calibrations were performed separately using a glycol standard (80% in DMSO-d_6). The uncertainties in temperature measurements are ±1 K. Rate constants were derived from spectra by a full line-shape analysis using gNMR ver. 4.1.2 software (Cherwell Scientific). Standard deviations of the activation parameters were calculated taking the temperature uncertainty and the rate constant uncertainty into account.

Acknowledgment. We thank Ms. B. Simonsen (The Danish University of Pharmaceutical Sciences) for technical assistance, Ms. K. Petersen (Department of Chemistry, University of Copenhagen) for acquiring mass spectra of compounds **7**−**9**, and Dr. L. Bolt Jørgensen (Institute of Biology, University of Copenhagen) for plant material of *D. hebecarpa*. NMR equipment used in this work was purchased via grants from "Apotekerfonden af 1991", Copenhagen, and The Danish University of Pharmaceutical Sciences.

Supporting Information Available: Tables with correlations observed in COSY, NOESY or ROESY, HSQC, and HMBC spectra of **3**, **4**, **7**, **8**, and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Rehfeldt, A. G.; Schulte, E.; Spener, F. *Phytochemistry* **1980**, *19*, 1685−1689.
(2) Saleh, N. A. M.; El Sherbeiny, A. E. A.; El Sissi, H. I. *Qual. Plant. Mater. Veg.* **1969**, *17*, 384−394.
(3) Abdel-Fattah, A. F.; Zaki, D. A.; Edrees, M. *Qual. Plant.−Plant Foods Human Nutr.* **1975**, *24*, 311−316.
(4) Liu, H. I.; Hwang, I. H. *Zhonghua Nongye Yanjiu* **1991**, *40*, 280−290.
(5) Geyid, A.; Abebe, A.; Debella, A.; Makonnen, Z.; Aberra, F.; Teka, F.; Kebede, T.; Urga, K.; Yersaw, K.; Biza, T.; Mariam, B. B.; Guta, M. *J. Ethnopharmacol.* **2005**, *97*, 421−427.
(6) Stærk, D.; Witt, M.; Oketch-Rabah, H. A.; Jaroszewski, J. W. *Org. Lett.* **2003**, *5*, 2793−2796.
(7) Païs, M.; Rattle, G.; Sarfati, R.; Jarreau, F.-X.; Janot, M. M. *C. R. Acad. Sci. Paris* **1968**, *263*, 37−40.
(8) Lefebvre-Soubeyran, O. *Acta Crystallogr.* **1976**, *B32*, 1305−1310.
(9) Wasserman, H. H.; Berger, G. D.; Cho, K. R. *Tetrahedron Lett.* **1982**, *23*, 465−468.
(10) Crombie, L.; Haigh, D.; Jones, R. C. F.; Mat-Zin, A. R. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2047−2054.
(11) Drakenberg, T.; Forsen, S. *J. Chem. Soc., Chem. Commun.* **1971**, 1404−1405.
(12) Fischer, G. *Chem. Soc. Rev.* **2000**, *29*, 119−127.
(13) Tchaicheeyan, O. *FASEB J.* **2004**, *18*, 783−789.
(14) Scherer, G.; Kramer, M. L.; Schutkowski, M.; Reimer, U.; Fischer, G. *J. Am. Chem. Soc.* **1998**, *120*, 5568−5574.
(15) Bienz, S.; Detterbeck, R.; Ensch, C.; Guggisberg, A.; Häusermann, U.; Meisterhans, C.; Wendt, B.; Werner, C.; Hesse, M. In *The Alkaloids, Chemistry and Biology*; Cordell, G. A., Ed.; Academic Press: Amsterdam, 2002; pp 83−338.
(16) Ekabo, O. A.; Farnsworth, N. R.; Santisuk, T.; Reutrakul, V. *J. Nat. Prod.* **1993**, *56*, 699−707.
(17) Zapesochnaya, G. G.; Kurkin, V. A.; Braslavskii, V. B.; Filatova, N. V. *Chem. Nat. Compds.* **2002**, *38*, 314−318.
(18) Thieme, H.; Richter, R. *Pharmazie* **1966**, *21*, 251.
(19) Pearl, I. A.; Darling, S. F. *Phytochemistry* **1971**, *10*, 3161−3166.
(20) Erickson, R. L.; Pearl, I. A.; Darling, S. F. *Phytochemistry* **1970**, *9*, 857−863.
(21) Pearl, I. A.; Darling, S. F. *J. Org. Chem.* **1962**, *27*, 1806−1809.
(22) Thieme, H. *Pharmazie* **1964**, *19*, 471−475.
(23) Pearl, I. A.; Darling, S. F. *Phytochemistry* **1970**, *9*, 853−856.
(24) Bienz, S.; Bisegger, P.; Guggisberg, A.; Hesse, M. *Nat. Prod. Rep.* **2005**, *22*, 647−658.
(25) Chase, M. W.; Zmarzty, S.; Lledo, M. D.; Wurdack, K. J.; Swensen, S. M.; Fay, M. F. *Kew Bull.* **2002**, *57*, 141−181.
(26) APGII *Bot. J. Linnean Soc.* **2003**, *141*, 399−436.
(27) Ekabo, O. A.; Farnsworth, N. R.; Santisuk, T.; Reutrakul, V. *Phytochemistry* **1993**, *32*, 747−754.

NP060204E