Conformational Analysis of Febrifugines and Halofuginones in Organic Solvents

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The stereostructures of anticoccidial agents, febrifugine and halofuginone, as well as the corresponding *cis*-isomers, in organic solvents were determined through NMR analyses, isomerization reactions and AM1 molecular orbital calculations.

Key words *Plasmodia*; *Coccidia*; anticoccidial agent; febrifugine; halofuginone; AM1 molecular orbital calculation

During our search for anticoccidial agents from natural drugs and related plants, we isolated *cis*-febrifugine (**1**) and *trans*-febrifugine (**2**) from *Hydrangea macrophylla* (Saxifragaceae); these compounds had already been isolated from *Dichroa febrifuga* (antimalarial natural drug) by Koepfli *et al.* in 1947[1] and from *Hydrangea umbellata* by Ablondi *et al.* in 1952.[2] Synthesis of febrifugines was first achieved in 1952 by Baker *et al.*,[3] who later reported that febrifugine obtained from *D. febrifuga* corresponds to the synthetic *cis*-febrifugine (**1**), whereas isofebrifugine corresponds to the synthetic *trans*-febrifugine (**2**).[4] Afterwards, Barringer *et al.*[5] established through detailed ^{1}H-NMR analysis that the assignments by Baker *et al.* should be reversed, *i.e.*, febrifugine has a *trans*-orientation and isofebrifugine has a *cis*-configuration. Thus, the absolute configurations of febrifugines were established.

As regards anticoccidial activities on chickens, the *cis*-isomer (**1**) was reported to be *ca.* 10-fold less active than the *trans*-isomer (**2**).[5] The *cis*-isomer (**1**) isolated by us, however, did not show any activity even at 25 times the effective dose (Index of Efficacy = 100% at 3 ppm) of the *trans*-isomer (**2**).[6] Additionally, an extensive denaturation was observed at the cytoplasmic membrane and protoplasm of *Plasmodia* collected from mice fed the *trans*-isomer (**2**), but not in *Plasmodia* collected from mice fed the *cis*-isomer (**1**).[7]

Baker *et al.*[4b] suggested on the basis of the IR spectrum (KBr) in the crystalline state that isofebrifugine (*cis*-febrifugine (**1**)) should have a hemi-ketal form. However, it seemed to us that there would be a difference in the types and ratios of conformational isomers between *cis*- and *trans*-febrifugines (**1**, **2**) in organic solvents, depending on their polarities. Such a difference might be correlated with the differential efficacy of the *cis*- and *trans*- isomers (**1**, **2**) against *Plasmodia*.

This paper deals with a detailed conformational analysis of *cis*- and *trans*-febrifugines (**1**, **2**) and their 6-bromo, 7-chloro derivatives, *cis*- and *trans*-halofuginones (**3**, **4**), in organic solvents through NMR measurements and examination of isomerization reactions, as well as AM1 molecular orbital calculations.

Results and Discussion

cis- and *trans*-Febrifugines (**1**, **2**) were isolated from *Hydrangea macrophylla*.[6] However, they were obtained only in small amounts, and were not easy to handle due to their instability. Thus, the synthetic 6-bromo, 7-chloro derivatives of these compounds, *cis*- and *trans*-halofuginones (**3**, **4**) (both racemic forms),[8] which are expected to be substitutes for febrifugines (**1**, **2**), were isolated from Stenorol[9] (Roussel Uclaf. Co. Ltd.). Comparison of the NMR spectra showed that the signal patterns and chemical shifts of *cis*- and *trans*-febrifugines (**1**, **2**) are closely similar to those of *cis*- and *trans*-halofuginones (**3**, **4**), except for the quinazolinone moieties, in $CDCl_3$, dimethyl sulfoxide (DMSO)-d_6 and acetate buffer (CD_3COOD, CD_3COONa and D_2O, pH 3.9). As an example, the ^{1}H- and ^{13}C-NMR spectral data of **1**, **2**, **3** and **4** are listed in Tables 1 and 2, respectively. We therefore carried out the NMR analyses by using *cis*- and *trans*-halofuginones (**3**, **4**), and, when necessary, *cis*- and *trans*-febrifugines (**1**, **2**) as well.

Conformation in $CDCl_3$ The ^{13}C-NMR spectrum of *cis*-halofuginone (**3**) was compared with that of *trans*-febrifugine (**2**) in place of *trans*-halofuginone (**4**), since the spectrum of **4** could not be taken owing to its poor solubility in $CDCl_3$. The ^{13}C-NMR spectrum of **3** lacks the keto-carbonyl carbon signal appearing at δ 202.66 in *trans*-febrifugine (**2**), but instead, has a hemi-ketal carbon signal at δ 105.18 . Furthermore, considering the downfield shift of the carbonyl carbon (δ 161.47) in the quinazolinone of *cis*-febrifugine (**1**) by 0.48 ppm from that (δ 160.99) of *trans*-febrifugine (**2**), it is likely that *cis*-febrifugine (**1**) and thus *cis*-halofuginone (**3**) have a stable hemi-ketal ring whose hydroxy group is hydrogen-bonded to the carbonyl group in the quinazolinone ring, as shown in Chart 1. Decoupling experiments in **3** indicated that the piperidine ring has a chair conformation in which 3″-H is equatorial ($J_{3'',2''} = 3.1$ Hz; $J_{3,4ax''}$ and $J_{3'',4eq''} < 2.0$ Hz), and 2″-H is axial ($J_{2'',3''} = 3.1$ Hz).

The stereochemistry at the hemi-ketal carbon in *cis*-halofuginone (**3**) was deduced from the following result. In the ^{1}H-NMR spectrum of **3**, 3′-Ha at δ 2.06 is coupled ($J = 3.7$ Hz) to 2″-H at δ 3.32, whereas 3′-Hb at δ 1.85 is

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not, indicating that 3′-Hb is oriented at an angle of almost 90° to 2″-H. Furthermore, nuclear Overhauser effects (NOEs) with larger enhancements at 3′-Hb than at 3′-Ha on irradiation at either 1′-Ha or 1′-Hb demonstrated that 3′-Hb is closer than is 3′-Ha to both 1′-Ha and 1′-Hb. Hence, 3′-Hb is *trans*-oriented to the hydroxy group at the 2′-hemi-ketal carbon in **3**. It was therefore concluded that the 2′-hemi-ketal carbon is *R*-oriented in **1**, as shown in Chart 1. The ^{1}H-NMR spectrum of *trans*-halofuginone (**4**) showed a singlet-like signal at δ 4.84 due to 1′-methylene protons instead of the AB type signals at δ 4.18 and 4.38 in the *cis*-isomer (**3**). In view of the existence of a keto carbonyl carbon at δ 202.66 instead of the hemi-ketal carbon in the ^{13}C-NMR spectrum of *trans*-febrifugine (**2**), it is most likely that *trans*-halofuginone (**4**) and hence *trans*-febrifugine (**2**) possess a ketonic side chain (designated as keto form), as shown in Chart 1. The conformation of the piperidine ring in **4** was also confirmed to be a chair form in which 3″-H is axial ($J_{3'',2''}=8.7$ Hz; $J_{3'',4ax''}=8.7$ Hz; $J_{3'',4eq''}=4.5$ Hz), and 2″-H is axial ($J_{2'',3''}=8.7$ Hz).

Table 1. ^{1}H- and ^{13}C-NMR Data[a] for Febrifugines in $CDCl_3$

Numbering	*cis*-Febrifugine (**1**)		*trans*-Febrifugine (**2**)	
	Proton	Carbon	Proton	Carbon
2	8.30 (s)	148.21	8.32 (s)	148.18
4		161.47		160.99
1′	4.46 (AB, 13.9) 4.14 (AB, 13.9)	49.0	4.86 (br s)	54.85
2′		105.33		202.66
3′	2.08 (dd, 13.2, 3.7, Ha) 1.86 (d, 13.2, Hb)	42.8	3.11 (dd, 15.9, 4.7, Ha) 2.64 (dd, 15.9, 7.4, Hb)	44.01
2″	3.27 (br t, 3.7)	55.51	2.87 (ddd, 8.6, 7.6, 4.7)	60.15
3″	3.88 (br d, 2.4)	77.23	3.28 (ddd, 10.1, 9.4, 4.5)	72.21
4″	1.98—2.23 (m, Heq) 1.38—1.65 (m, Hax)	26.85	2.03—2.14 (m, Heq) 1.23—1.42(m, Hax)	34.44
5″	1.65—1.98 (m, Heq) 1.38—1.65 (m, Hax)	20.12	1.67—1.78 (m, Heq) 1.43—1.61 (m, Hax)	25.61
6″	3.00 (br d, 9.4 Heq) 2.52 (br td, 11.2, 2.0, Hax)	44.47	2.96 (br d, 12.1 Heq) 2.58 (td, 12.1, 2.7, Hax)	45.95

a) Multiplicities and coupling constants (Hz) in parentheses.

Table 2. ^{1}H- and ^{13}C-NMR Data[a] for Halofuginones in $CDCl_3$

Numbering	*cis*-Halofuginone (**3**)		*trans*-Halofuginone (**4**)[b]
	Proton	Carbon	Proton
2	8.28 (s)	149.68	8.03 (s)
4		159.95	
1′	4.38 (AB, 13.9) 4.18 (AB, 13.9)	50.19	4.84 (br s)
2′		105.18	
3′	2.06 (dd, 13.1, 3.7, Ha) 1.85 (d, 13.1, Hb)	43.53	3.10 (dd, 14.4, 4.7, Ha) 2.62 (dd, 14.4, 7.3, Hb)
2″	3.32 (br td, 3.7, 3.1)	55.68	2.87 (ddd, 8.7, 7.3, 4.7)
3″	3.90 (br d, 3.1)	77.82	3.23 (br td, 8.7, 4.5)
4″	1.96—2.25 (m, Heq) —	26.80	2.03—2.13 (m, Heq) 1.29—1.42 (m, Hax)
5″	1.66—1.96 (m, Heq) 1.43—1.66 (m, Hax)	20.09	1.68—1.79 (m, Heq) 1.42—1.68 (m, Hax)
6″	3.00 (br d, 11.2, Heq) 2.54 (br td, 11.2, 2.0, Hax)	44.53	2.92—3.01 (br d, Heq) 2.58 (td, 12.1, 3.0, Hax)

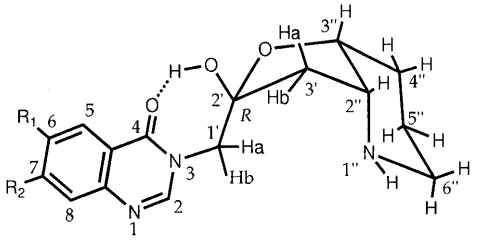
a) Multiplicities and coupling constants (Hz) in parentheses. *b*) The ^{13}C-NMR spectrum could not be taken owing to poor solubility of the compound in $CDCl_3$.

Conformation in DMSO-d_6 Table 3 shows the ^{1}H- and ^{13}C-NMR spectral data of **3** and **4** in DMSO-d_6. The spectum of *cis*-halofuginone (**3**) showed two sets of AB type signals (each $J_{AB}=13.7$ Hz) at δ 4.06 and 4.38 and at δ 4.09 and 4.23, attributed to 1′-methylene protons in hemi-ketal forms, respectively. Additionally, it showed a singlet-like signal at δ 5.00 due to 1′-methylene protons in a keto form.

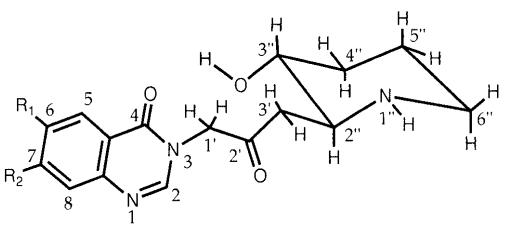
The ^{13}C-NMR spectrum showed signals originating from three forms, including hemi-ketal carbons at δ 103.86 and 104.58 and a keto carbonyl carbon at δ 202.93. From the signal intensities of the 1′-methylene protons, it was deduced that the keto form and 2′-epimeric hemi-ketal forms exist in about (1 : 1 : 1) ratio in DMSO-d_6.

trans-Halofuginone (**4**) showed a singlet at δ 5.00 due to 1′-methylene protons and a keto carbonyl carbon at δ 203.39, but did not exhibit any signal due to a hemi-ketal form. Thus, it follows that *trans*-halofuginone (**4**) exists in a keto form in DMSO-d_6.

Conformation in the Acetate Buffer Table 4 shows the ^{1}H- and ^{13}C- NMR spectral data of **3** and **4** in the acetate buffer. The ^{1}H-NMR spectrum of *cis*-halofuginone (**3**) showed an intense singlet at δ 4.78 due to 1′-methylene protons of a keto form. Furthermore, the ^{13}C-NMR spectrum showed weak singlets at δ 102.52 and 104.16 due to hemiketal carbons, besides an intense signal at δ 201.94 due to a keto carbonyl carbon. These carbon signals indicated that there exists a keto form as well as 2′-epimeric hemi-ketal forms in the acetate buffer, as in DMSO-d_6. The intense singlet at δ 4.78 in the ^{1}H-NMR spectrum indicated that the keto form is the major conformer. The NMR spectra of *trans*-halofuginone (**4**) indicated that it exists in a keto form in the acetate buffer, as in $CDCl_3$



1 R_1=R_2=H, *cis*-febrifugine



2 R_1=R_2=H, *trans*-febrifugine

3 R_1=Br, R_2=Cl, *cis*-halofuginone

4 R_1=Br, R_2=Cl, *trans*-halofuginone

Chart 1

Table 3. ^{1}H- and ^{13}C-NMR Data[a] for Halofuginones in DMSO-d_6

Numbering	*cis*-Halofuginone (**3**)		*trans*-Halofuginone (**4**)	
	Proton	Carbon	Proton	Carbon
2	8.25 (s)	149.67, 150.33, 150.79	8.22 (s)	149.66
4		158.57, 159.02, 159.57		158.57
1′	4.06, 4.38 (AB, 13.7)	49.87, 51.22	5.00 (s)	54.72
	4.09, 4.23(AB, 13.7) 5.00 (br s)	54.66		
2′		103.86, 104.58, 202.93		203.39
3′	1.67 (d, 13.2, Ha)	41.62, 42.70	2.39 (dd, 15.0, 8.7, Ha)	43.63
	1.96 (dd, 13.2, 4.2, Hb)	43.53	2.98 (dd, 15.0, 3.8, Hb)	
2″	3.05—2.97 (m)	55.00, 55.62	2.66 (td, 8.7, 3.8)	60.00
	3.16—3.09 (m)	55.97		
3″	3.73 (br d, 2.4)	65.22, 74.36	*ca.* 3.00	70.65
	3.94 (br d, 3.2) 4.51 (br d, 4.6)	76.60		
4″	—	25.48, 26.42, 30.86	—	34.14
5″	—	20.03, 20.18, 20.65	—	25.75
6″	2.81 (br d, 13.7, Heq)	43.77, 44.50	2.80 (br d, 12.0, Heq)	45.50
	—	45.48	2.36 (td, 12.0, 2.5, Hax)	

a) Multiplicities and coupling constants (Hz) in parentheses.

Table 4. ^{1}H- and ^{13}C-NMR Data[a] for Halofuginones in Acetate Buffer

Numbering	*cis*-Halofuginone (**3**)		*trans*-Halofuginone (**4**)	
	Proton	Carbon	Proton	Carbon
2	7.54 (s), 7.58 (s) 7.59 (s)	—	7.81 (s)	148.90
4		160.37, 160.80	—	160.33
1′	4.78 (s)	—	—	55.12
2′	—	102.52, 104.16, 201.94		201.96
3′	—	39.37	—	38.88
2″	3.45 (br s)	54.92, 55.15	3.22 (d, 9.8)	56.24
	3.55 (d, 2.7)	55.54, 55.62		
	3.66 (d, 3.6)			
3″	3.78 (br s)	64.25, 72.92	3.45 (td, 9.8, 4.0)	67.32
	3.99 (d, 2.7)	75.89, 76.49		
	4.17 (d, 3.6)			
4″	1.74—1.87 (m, Heq)	21.21, 23.79	1.84 (td, 9.8, 4.0, Heq)	30.8
	1.37—1.59 (m, Hax)	28.46	1.21—1.37 (m, Hax)	
5″	1.59—1.74 (m, Heq)	16.20	1.65—1.78 (m, Heq)	20.11
	1.37—1.59 (m, Hax)		1.37—1.55 (m, Hax)	
6″	3.08 (d, 12.4)	43.20, 44.78	3.07 (td, 12.9, 3.2, Heq)	43.93
	2.78 (td, 12.4, 3.4)	44.86	2.74 (td, 12.9, 3.2, Hax)	

a) Multiplicities and coupling constants (Hz) in parentheses.

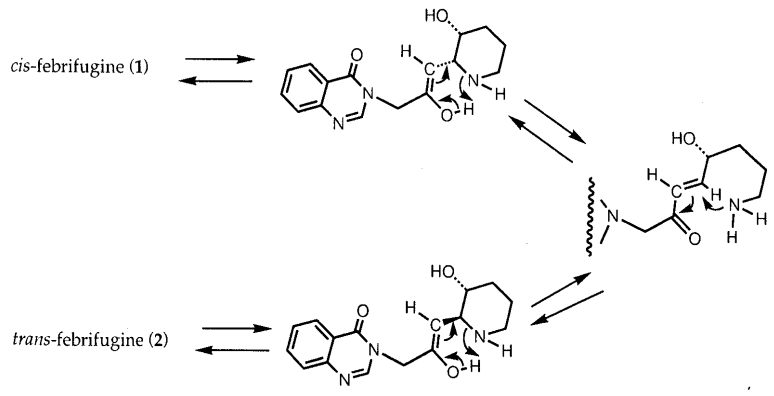


Chart 2

and DMSO-d_6.

Isomerization of Halofuginones It is known that *cis*- and *trans*-febrifugines (**1**, **2**) can be interconverted through refluxing in organic solvents[10] according to the mechanism[11] shown in Chart 2. In order to examine in detail the stability of *cis*- and *trans*-halofuginones (**3**, **4**) in solutions, isomerization reactions were carried out in $CHCl_3$ and EtOH. On refluxing in EtOH solution, *cis*- and *trans*-halofuginones were 70% and 30% isomerized to the counterparts, respectively. On refluxing in $CHCl_3$ solution, however, whereas the *trans*-isomer (**4**) was 60% isomerized to the *cis*-isomer (**3**), the *cis*-isomer (**3**) remained as such. This result supports the aforementioned proposal that *cis*-halofuginone (**3**) is stabilized as the hemi-ketal form with intramolecular hydrogen bonding in the non-polar solvent, $CHCl_3$.

Conformational Analyses In $CDCl_3$ *cis*-halofuginone (**3**) and hence, *cis*-febrifugine (**1**), were found to exist as the single hemi-ketal form (*R* chirality at C-2′ in the case of **1**). However, in the more polar solvents (DMSO-d_6, and the acetate buffer), the keto form exists as well as the 2′-epimeric hemi-ketal forms. In contrast, *trans*-halofuginone (**4**) and, hence, *trans*-febrifugine (**2**) exist in the keto form regardless of the solvent polarity.

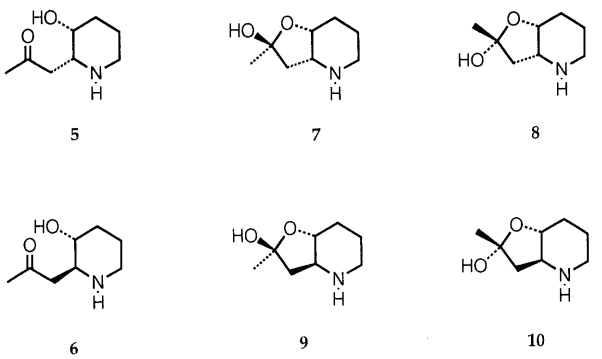
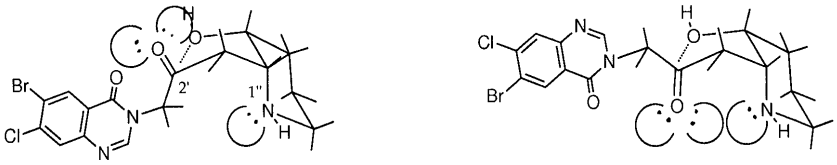


Chart 3

Table 5. Heats of Formation of the Most Stable Conformations of the Model Compounds Obtained by AM1 Molecular Orbital Calculations

Model compound	Heat of formation (kcal/mol)
5	−108.33
6	−109.19
7	−111.46
8	−111.87
9	−107.23
10	−106.41



(favored) (disfavored)

transition state A transition state B

Chart 4

The preponderant formation of the hemiketal form of *cis*-halofuginone (**3**) in $CDCl_3$ was rationalized by means of semi-empirical molecular orbital calculations on model compounds in the following way. The most stable conformations of the model compounds of the keto (**5**, **6**) and hemi-ketal (**7**—**10**) forms *in vacuo* (Chart 3) were obtained by AM1 molecular orbital calculations.[12] Table 5 shows the heats of formation of the respective most stable conformations. It indicates that the hemi-ketal forms (**7**, **8**) are more stable than the corresponding keto form **5** in the case of the *cis*-configuration, while the keto form **6** is more stable than the corresponding hemi-ketal forms (**9**, **10**) in the case of the *trans*-orientation. Therefore, since the interconversion between the keto and hemi-ketal forms, which takes place upon changing the solvents, is considered to be thermodynamically controlled, the *cis*-hemi-ketal forms (**7**, **8**) can predominate over the corresponding keto form **5**, while the *trans*-keto form **6** can exist preferentially over the corresponding hemi-ketal forms (**9**, **10**). The origin of the stereochemistry (*R*-chirality in **1**) in the *cis*-hemi-ketal form in $CDCl_3$ is not clear. It might be due to the anomeric effect. However, in view of the exclusive formation of *R*-chirality, it could be considered, as shown in Chart 4, that the transition state A is favored as a consequence of a dipole–dipole repulsive interaction between the 2′-keto and C-2″–NH groups or an electronic repulsion between the lone pairs of both groups that destabilizes B relative to A. In contrast, in the more polar solvents DMSO-d_6 and acetate buffer, such a repulsive interaction would be reduced by coordination of these solvents to the said groups in *cis*-form.

In conclusion, *trans*-halofuginone (**4**) and *trans*-febrifugine (**2**), which showed a strong anticoccidial activity, have the keto form regardless of solvent polary. In contrast, *cis*-halofuginone (**3**) and *cis*-febrifugine (**1**), the latter exhibiting no activity even at a concentration 25-fold higher than the effective concentration of **2**, adopt the hemi-ketal form exclusively in a non-polar solvent and a mixture of keto and hemi-ketal forms in polar solvents. The intriguing possibility arises that the difference in conformational behavior between the *cis*- and *trans*-isomers is responsible for the difference in anticoccidial activity.

Further study on the structure–activity relationship is under way using *Coccidia*.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 polarimeter, IR spectra with a Shimadzu IR-435 IR spectrometer, and ultraviolet (UV) spectra with a Hitachi 323 recording spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM FX-200FT, Varian-XL-300, Bruker-AC-300P and Bruker-AM-600 instruments. The acetate buffer (pH 3.9) was prepared by mixing 2 ml of 0.3 M CD_3COOD in D_2O and 0.5 ml of 0.17 M CD_3COONa in D_2O. Chemical shifts are given in ppm with tetramethylsilane as an internal standard ($CDCl_3$, DMSO-d_6) or external standard (the acetate buffer), and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublets of doublets and AB = AB quartet. NOE enhancements were determined by differential NOE techniques. All thin layer chromatography (TLC) and preparative TLC procedures were performed on Kieselgel 60 F_{254} (Merck). The molecular dynamics and energy minimization were performed with the Discover program (Biosym Technologies) using the CVFF force field. The AM1 molecular orbital calculations were carried out using the MOPAC program within the INSIGHT II package (Biosym Technologies). All calculations were made on a IRIS Crimson computer (Silicon Graphics).

Isolation of *cis*- and *trans*-Halofuginones (3, 4) from Stenorol Stenorol purchased from Japan Scientific Feed Association (containing halofuginones 0.6 g in 100 g) (700 g) was suspended in H_2O (820 ml) and filtered. The insoluble material was washed with H_2O (1460 ml). The combined filtrate and washings were saturated with NaCl, adjusted to pH 8.5 with 20% NaOH and extracted with $CHCl_3$ (1 l × 5). The $CHCl_3$ extract, after having been dried with K_2CO_3, was concentrated *in vacuo* to give halofuginones (1.41 g) as a solid (83.6%). Repeated recrys-

tallizations from EtOH afforded *trans*-halofuginone (**4**) (860 mg) as colorless needles. The mother liquor from the recrystallizations yielded a residue (252 mg), which was subjected to PLC ($CHCl_3$–MeOH–H_2O, 75 : 25 : 2, two developments). The main band was scraped off and suspended in 0.1 N HCl (adjusted to pH 2.0) for 15 min, under ice-cooling. The suspension was saturated with NaCl, adjusted to pH 8.5 with 20% Na_2CO_3 and then extracted with $CHCl_3$. The $CHCl_3$ extract afforded *cis*-halofuginone (**3**) (96.7 mg) as colorless needles.

trans-Halofuginone (**4**): mp 190—191 °C. *Anal.* Calcd for $C_{16}H_{17}BrClN_3O_3$: C, 46.34: H, 4.13; Br, 19.30; Cl, 8.55; N, 10.13; O, 11.60. Found: C, 46.02; H, 3.94; Br, 19.52; Cl, 8.66; N, 9.85; O, 11.44. UV λ_{max} (EtOH) nm (log ε): 242 (4.62), 276 (3.92), 285 (3.90), 314 (3.46), 327 (3.42). IR (KBr) cm^{-1}: 3280 (OH, NH), 3080, 2930, 2830, 1715 (C=O), 1680 (C=O), 1600, 1442, 1263, 1220, 1100, 1080, 738, 690.

cis-Halofuginone (**3**): mp 137—141 °C. *Anal.* Calcd for $C_{16}H_{17}BrClN_3O_3$: C, 46.34; H, 4.13; Br, 19.30; Cl, 8.55; N, 10.13; O, 11.60. Found: C, 46.35; H, 4.19; Br, 19.16; Cl, 8.50; N, 9.97; O, 11.52. UV λ_{max} (EtOH) nm (log ε): 243 (4.61), 277 (3.92), 285 (3.91), 315 (3.48), 328 (3.41). IR (KBr) cm^{-1}: 3300 (OH, NH), 3050, 2930, 2850, 1680 (C=O), 1600, 1445, 1260, 1180, 1090, 1055, 650.

Isomerization of *trans*-Halofuginone (4) in EtOH and in $CHCl_3$ *trans*-Halofuginone (**4**) (10.7 mg) was refluxed in EtOH (4 ml) for 3 h. The reaction was followed by measuring the UV absorption (at 262 nm) of spots on TLC ($CHCl_3$–MeOH–H_2O, 75 : 25 : 2) with a chromatoscanner. The reaction mixture (*trans* : *cis* = 2 : 1 on TLC) was concentrated *in vacuo* and subjected to PLC in the same way as for the isolation of *cis*-halofuginone (**3**) from Stenorol, giving the *trans*-isomer (**4**) (3.9 mg) and *cis*-isomer (**3**) (2.4 mg). *trans*-Halofuginone (**4**) (11.2 mg) was refluxed in $CHCl_3$ (4 ml) for 2 h in the same way as mentioned above. PLC of the reaction mixture (*trans* : *cis* = 5 : 7 on TLC) afforded the *trans*-isomer (**4**) (3.2 mg) and *cis*-isomer (**3**) (6.1 mg).

Isomerization of *cis*-Halofuginone (3) in EtOH and $CHCl_3$ *cis*-Halofuginone (**3**) (9.5 mg) was refluxed in EtOH (4 ml) for 2 h in the usual way. PLC of the mixture (*trans* : *cis* = 2 : 1 on TLC) furnished the *trans*-isomer (**4**) (6.0 mg) and *cis*-isomer (**3**) (2.3 mg). *cis*-Halofuginone (**3**) (3.6 mg) was refluxed in $CHCl_3$ (2 ml) for 3 h, leading to recovery of the starting *cis*-isomer (**3**) (confirmed by the ^{1}H-NMR spectrum).

Conformational Searches i) Model Compounds **5** and **6**: The conformations were generated by systematically rotating the rotatable bonds by a 60 degree increment. After the conformations had been optimized with molecular mechanics, each conformer was matched to every other over the cartesian coordinates of the heavy atoms and the hydrogen atoms bonded to the hetero atoms using a least-squares method. In the case that the RMS deviation between two conformers was greater than 0.2 Å, they were considered to be different. All the unique conformers were optimized using AM1 molecular orbital calculations.

ii) Model Compounds **7**—**10**: A hundred initial conformations were generated by randomizing the atom coordinates of the molecule. Each structure was first minimized and then heated to 1000 K over 23 ps while progressively increasing the covalent constants (to twice their full value) and the nonbonded term (to 25% of its full value). The structures were then cooled gradually to 300 K over 10 ps while the nonbonded term was increased to its full value. The final minimization completed the calculation, using a force field in which all the force constants took their full values. Until the final minimization, the nonbonded potential was reduced to a purely repulsive term (quartic). Each resultant conformer was matched to every other over the cartesian coordinates of heavy atoms and the hydrogen atoms bonded to the hetero atoms using a least-squares method. In the case that the RMS deviation between two conformers was greater than 0.2 Å, they were considered to be different. All the unique conformers were optimized using AM1 molecular orbital calculations.

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