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Structure of a new Usnic acid derivative from a deacylating Mannich reaction.

NMR studies supported by theoretical calculations of chemical shifts.

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Abstract

In a conventional Mannich reaction using piperidine, hydroxypiperidines, morpholine and N-methylpiperazine with usnic acid a deacetylation was observed resulting in a substitution at C-2, loss of an acetyl group and a Mannich base with a stabilized enol. The enol has a hydrogen bond to the nitrogen of the secondary amine. The structure was investigated by NMR and deuterium isotope effects on ^{13}C chemical shifts as well as with DFT calculations to study the changed hydrogen bond pattern. It was found that the hydrogen bond involving the OH-9 group in chloroform forms a strong hydrogen bond than in usnic acid itself and that this hydrogen bond becomes even stronger in the more polar solvent, dimethylsulfoxide. Tautomerism was observed in the Mannich base as demonstrated by deuterium isotope effects on chemical shifts. The position of the tautomeric equilibrium depends on the solvent and the position of the equilibrium governs the strength of the OH-9...O=C hydrogen bond.

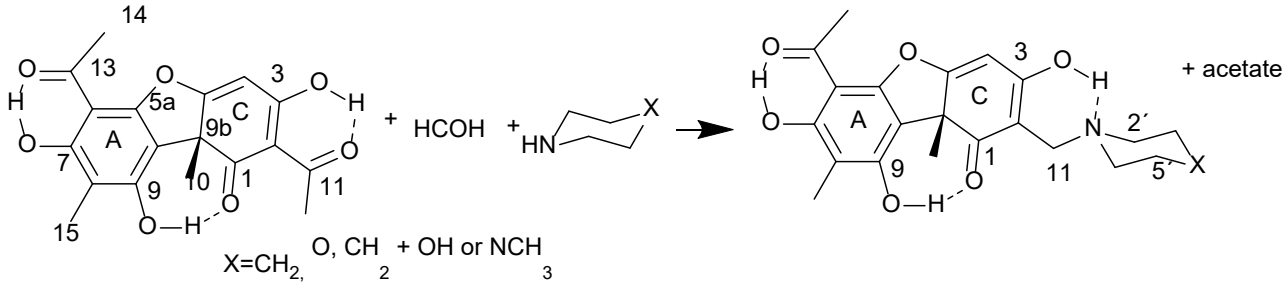
Keywords

DFT calculations, isotope effects on chemical shifts, Mannich reaction, deacylation, usnic acid

Introduction

Usnic acid has a complex hydrogen bonded structure showing tautomerism [1-3]. In addition, usnic acid is known to have many useful biological properties [4-8]. To improve on these properties a number of interesting derivatives of usnic acid has been synthesized [8-10]. However, the solubility of usnic acid in water is very low [11], so some of the efforts have aimed at overcoming this problem. The first pK_a value of usnic acid is as low as 4.4 [12]. The acidic proton is the OH-3 or that of H-2 if the latter exists at all. In order to search for compounds with better biological properties and better solubility a Mannich reaction was performed between usnic acid and piperidine or morpholine or 3- and 4-hydroxypiperidine or N-methylpiperazine to add an amino moiety and thereby increasing the solubility (the resulting products are numbered as **1**-**5**). This resulted in substitution at C-2, but was followed by an unusual step, a deacetylation leading to a Mannich base (Scheme 1). A deacylation has also been seen in an enzymatic reaction, but without a mechanism given [13]. Interestingly, a Mannich reaction done by reacting $[Me_2NCH_2]^+Cl^-$ with β-diketones yielded $[RCOCHCOR'CH_2NHMe_2]^+Cl^-$, that upon treatment with water lost the amino group [14]. A similar reaction was reported by Matsuda *et al.* [15].

The aim of the present study was to generate new usnic acid derivatives and to explore the changes in the hydrogen bond pattern or tautomerism as even relatively small changes, acetylation of the OH-9 group, led to a change in the tautomeric equilibrium [2].



Scheme 1. Mannich reaction. The usnic acid is the +-form. All compounds are numbered according to the scheme. Hydrogens and the OH groups have the same numbers as the carbons of which they are attached.

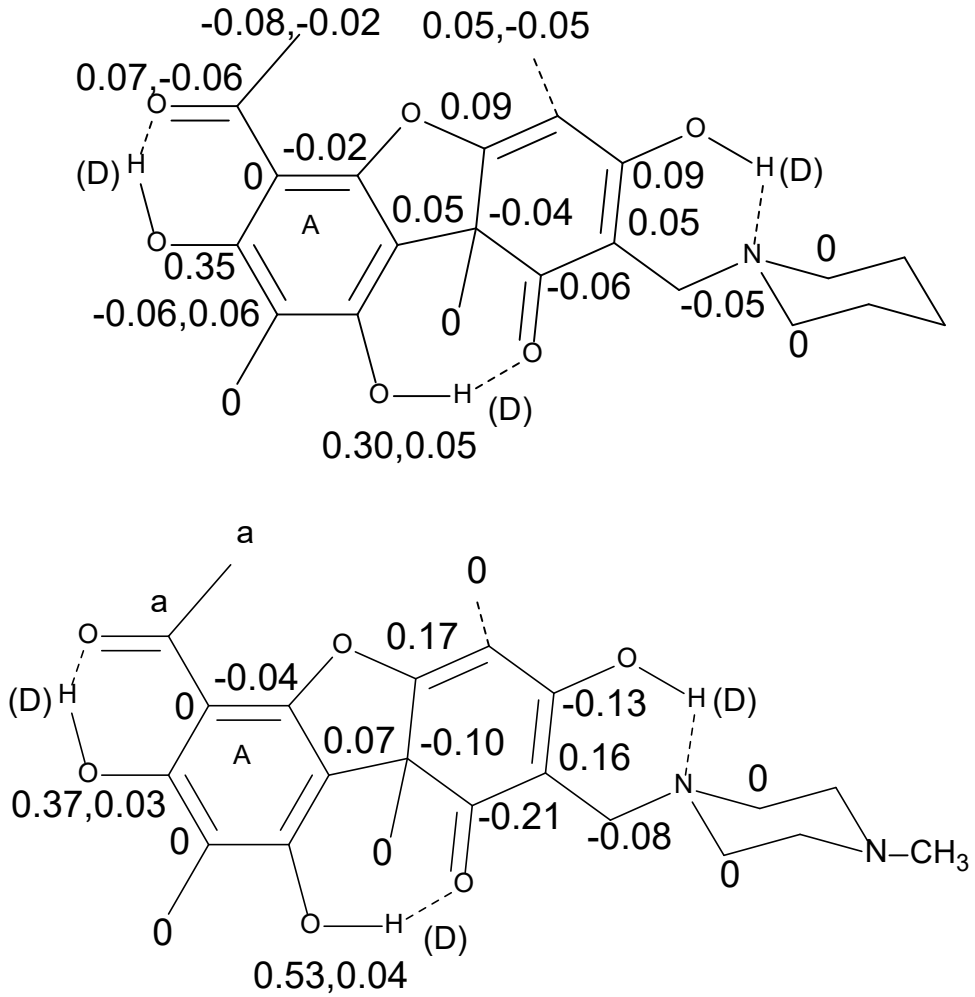
Results

Assignments

The ^{13}C NMR spectra were assigned based on HMBC spectra (for spectra see Supplementary mat.) as seen in Tables 1 and 2 for the product using piperidine in DMSO-d_6 or N-methylpiperazine in $CDCl_3$ as the secondary amine. The OH-7 and OH-9 protons can then be assigned based on the HMBC spectra. In case of the substituent at C-3 this is referred to as XH as the hydrogen may be either on O or on N (see later). The XH-3 could not be seen in the latter but could be observed in the former. Molecular weights for the five products made from piperidine (**1**), morpholine (**2**), 3-hydroxypiperidine (**3**), 4-hydroxypiperidine (**4**) or N-methylpiperazine (**5**) are: M+H, 400, 402, 416, 416 and 414 (see experimental for HRMS data) clearly showing loss of C_2H_3O which is equal to a CH_3CO group. This is confirmed in both the ^1H and ^{13}C spectra (see Table 1) as only three CH_3 resonances belonging to the usnic acid moiety are observed in the ^1H and ^{13}C spectra and one C=O resonance is "missing" from the ^{13}C NMR spectrum. The finding that the OH signal at ~19 ppm is not found indicates very strongly that the reaction has taken place at the C-ring and not in the A-ring. This can be further confirmed by the full analysis of the ^{13}C NMR spectrum. The cross peaks from H-4 in the HMBC spectrum (both one-bond and long-range correlations are seen, Table 1) define C-4, C-2, C-3, C-4a and C-9a. As C-9b is now assigned, the cross peak from 1.66 ppm defines H-10 (CH_3-group). The cross peaks from these hydrogens now assign C-1, so all carbons of the C-ring are assigned. As a cross peak is seen from H-11 (CH_2) to C-1, C-2 and C-3 and as C-3 have too high a chemical shift to simple be alkylated, the position of the C-11 attached to C-2 is secured. The chemical shift of 4.06 and 3.96 ppm for H-11 confirms that this is next to the nitrogen of the piperidine ring and also close to the center of chirality at C-9b.. The finding that cross peaks are seen from H-9 to C-1 and C-2 shows that OH-9 is forming a strong hydrogen bond to C-1, but also confirms the assignment of OH-9. Cross peaks from this furthermore assigns C-8 and C-9. Cross peaks from OH-7 assigns C-6, C-13 and C-15. Cross peak from H-14 assigns C-13. All carbons are now assigned. A comparison with ^{13}C chemical shifts of usnic acid (Table 1) furthermore shows rather similar similar shift of the A ring and also for the methyl groups still present in the molecule. The carbons of the heterocylic rings can be assigned based on comparison with model compounds. For compounds 2-4 the assignments are done in an analogous way and the chemical shifts are given in Table 3. Only small changes in the ^{13}C chemical shifts are seen for compounds **1**, **2** and **5** measured in $CDCl_3$.

The leaving acetyl group is apparently trapped by the amine as in the case of N-methylpiperazine, N-acetyl-N-methylpiperazine is identified.

The XH (X indicates either O or N) chemical shifts are given in Table 5. They are seen to vary dramatically with solvent for OH-9 and for XH-3 being absent in chloroform-*d* in some cases.
As seen in Tables 1-3 the chemical shifts are different for C-2′and C-6′due to the center of chirality at C-9b. However, much more important is the large chemical shift differences found for C-4′, C-3′and for C-2`(in addition to that mentioned above) for **4**. It is largest for C-4′. It is seen that the two ^{13}C signals for these pairs have the same intensity.
For **3** it must be considered that an new chiral center at C-3′ has been introduced. Also for **3** large chemical shift differences are found (See Table 4).
Deuterium isotope effects on chemical shifts are measured in **1** and **2** in $CDCl_3$ and for **5** in DMSO-d_6 as seen in Scheme 3. The deuteration is achieved by dissolving the compound in CH_3OD and evaporating the methanol. This treatment will lead to deuteration of all XH groups. The isotope effects on chemical shifts observed at the carbons C-7, C-13, C-14 are similar to what is observed for usnic acid itself, whereas the isotope effect on chemical shift at C-9 is clearly larger than for usnic acid [1], especially in DMSO-d_6. Isotope effects on chemical shifts may be of two types, intrinsic or equilibrium [1-3]. For hydrogen bonded cases, as in the present situation, the two-bond deuterium isotope effect on ^{13}C chemical shifts reflect the strength of the hydrogen bond and are transmitted in a way depending on the number of bonds and conjugation [1-3]. This fact means that isotope effects on chemical shifts are normally dominated by deuteration in the neighborhood. In case of equilibrium isotope effects the difference in chemical shifts between a nuclei in the two tautomers play an important role. [1-3] Calculated nuclear shieldings (selected) for the two forms (Scheme 3) are shown in Table 4. The differences for the two forms are seen clearly for carbons C-1, C-2 and C-3 and to some extent C-4.



Scheme 2. Deuterium isotope effect on ^{13}C chemical shifts in **1** and **5**.

Top: **1** in $CDCl_3$. Similar effects are observed in the morpholine derivative (**2**). The structure shown is only one of the tautomers, see Scheme 3.

Bottom: **5** in DMSO-d_6. a. refers to the fact that the methyl group is partially deuteriated so that the resonance is too complicated for analysis. The structure shown is only one of the tautomers, see Scheme 3.

Table 1. ^{1}H and ^{13}C chemical shifts for the piperidine derivative (**1**) in DMSO-d_6 and $CDCl_3$.

Carbon [a]	^{1}H chemical shifts ($CDCl_3$)	^{13}C C.S. ($CDCl_3$)	^{13}C C.S. (DMSO-d_6)	HSQC correlations [b]	HMBC correlations [c]
13		200.26 (201.8) [d]	200.84		
1		190.83 (198.1)	188.42		
3		187.49 (191.8)	186.04		
4a		176.51 (179.4)	174.81		
7	OH 13.38	163.25 (163.9)	162.68		C-6,C-7, C-13(w), CH_3-15(vw)
9	OH 13.31	159.90 (157.9)	160.12		C-8,C-9,C-1(vw),C-2(vw)
5a		156.11 (155.2)	156.37		
8		107.24 (109.4)	106.90		
9a		105.83 (104.0)	105.91		
6		101.13 (101.6)	102.48		
4	5.49	101.13 (98.4)	100.73	observed	C-2,C-3(vw),C-4a,C-9b
2		94.68 (105.3)	95.81		
CH_3-15	2.07	7.35 (7.6)	7.96	observed	C-7,C-8,C-9
CH_3-10	1.66	32.74 (32.2)	32.80		C-1,C-4a,C-9a,C-9b
CH_3-14	2.49	30.80 (31.1) [e]	31.27	observed	C-6,C-13
C-9b		54.67 (59.1)	53.50		
CH_2-11	4.06;3.96	53.87	50.75	observed	C-1,C-2,C3

C-2′,C6′	3.51;2.77	52.44; 52.93	52.14;51.98		
C-3′,C-5′	1.98;1.81	23.38; 23.69	22.50		
C-4′	1.80;1.50	22.28	21.85		

a. The numbering is the same as in usnic acid

b. Observed in the HMBC spectrum. HMBC refer to the spectrum in DMSO-d_6

c. Correlations observed for the ^{1}H chemical shift

d. Values in brackets are for usnic acid are from Ref. 16.

e. Assignment taken from Ref. 2 as the ^{1}H chemical shifts in usnic acid are too close to make an unambiguous assignment.

Table 2. ^{1}H and ^{13}C chemical shifts for the N-methylpiperazine derivative (**5**) in $CDCl_3$.

Carbon	^{1}H chemical shifts	^{13}C chemical shifts	HSQC correlations [a]	HMBC correlations [b]
13		200.40		
1		190.85		
3		187.53		
4a		176.69		
7	OH 13.29	163.24		C-6,C-7, C-13(w), CH_3-15(vw)
9	OH 13.34	158.84		C-8,C-9
5a		156.11		
8		107.35		
9a		105.70		
6		101.13		
4	5.59	101.20	observed	C-2,C-3(vw),C-4a,C-9b
2		93.97		
15	2.08	7.42	observed	C-7,C-8,C-9
10	1.69	30.78		C-1,C-4a,C-9a,C-9b
14	2.57	31.05	observed	C-6,C-13

C-9b		54.73		
CH_2-11		53.48		C-1,C-2,C3,C-2′
C-2′,C-6′	3.42;2.40	51.28;51.64		
C-3′, C-5′	2.97	51.93 [c]		
N-CH_3	2.37	45.40	observed	C-2′or C-3′

a. Observed in HMBC spectrum
b. Correlations observed for the ^{1}H chemical shift
c. Broad signal. Assignment of C-2′and C-3′is tentative, but based on the fact that C-2′is closest to the center of asymmetry.

Table 3. ^{1}H and ^{13}C chemical shifts for compounds **2**-**4**.

Carbon	^{1}H C.S. ($CDCl_3$) [a] **2**	^{13}C C.S. ($CDCl_3$) **2**	^{1}H C.S. (DMSO-d_6) **3**	^{13}C C.S. (DMSO-d_6) **3**	^{1}H C.S. (DMSO-d_6) **4**	^{13}C C.S. (DMSO-d_6) **4**
13		200.20		200.38		200.31
1		191.93		188.03		187.98
3	n.o.[b]	186.98	OH 8.56	186.00;185.57	OH 8.42	185.53
4a		176.74		174.38		174.33
7	OH 13.31	163.25	OH 13.42	162.20	OH 13.39	162.20
9	OH 13.11	158.74	OH 15.04; 14.94;14.91	159.58	OH 15.01	159.64
5a		156.01		155.88		155.89
8		107.46		106.38		106.40
9a		105.93		105.45		105.45
6		101.19		101.98		100.28
4	5.46	100.64	5.65	100.27	5.63	101.96

2		94.31		95.25;94.41		95.43;95.21
CH_3-15	2.07	7.37	1.93	7.48	1.91	7.42
CH_3-10	1.66	32.74	1.60	32.33	1.58	32.26
CH_3-14	2.47	30.85	2.62	30.80	2.61	30.83
C-9b		55.03		53.05		53.03;49.56
CH_2-11	4.13;4.00[c]	54.09	3.93;3.80[c]	50.97[d]	3.90; 3.75	50.03
C-2′,C-6`	3.42;3.03	51.76;51.37	e	2′ 56.31;55.97 6` 50.97[d]	3.25;3.04;2.84	49.47;49.29; 46.64;46.45
C-3′, C-5′	4.09; 3.85	64.18	~4.00,3.98[d]	3` 63.28;61.64 5′ 20.29;17.74	1.90;1.95;1.79;1.58	30.69;29.20
C-4′	-	-	e	30.81;28.05	3.62	63.79;60.12
OH	-	-	OH-3` 5.34;5.28		OH-4` 4.91[f];4.84[g]	

a. The values in DMSO-d_6 are only slightly different except for OH-3 and OH-9 (See Table 5).

b. The OH signal is observed at 8.93 ppm in DMSO-d_6

c. The coupling constant is 12.2 Hz

d. Overlapping

e. Resonances are observed, but not assigned.

f. Further split into two with a splitting of 0.008 ppm

g. Further split into two with a splitting of 0.006 ppm

Table 4. Calculated nuclear shieldings [a] for selected carbons of the two tautomeric forms of **1**.

Carbon	OH-form	NH-form [b]
1	2.03	8.23
2	96.54	104.33
3	19.65	10.66
4	98.31	92.65
4a	17.07	18.03
CH_2	135.41	131.68

a. Nuclear shieldings are "opposite" both in magnitudes and signs of chemical shifts.

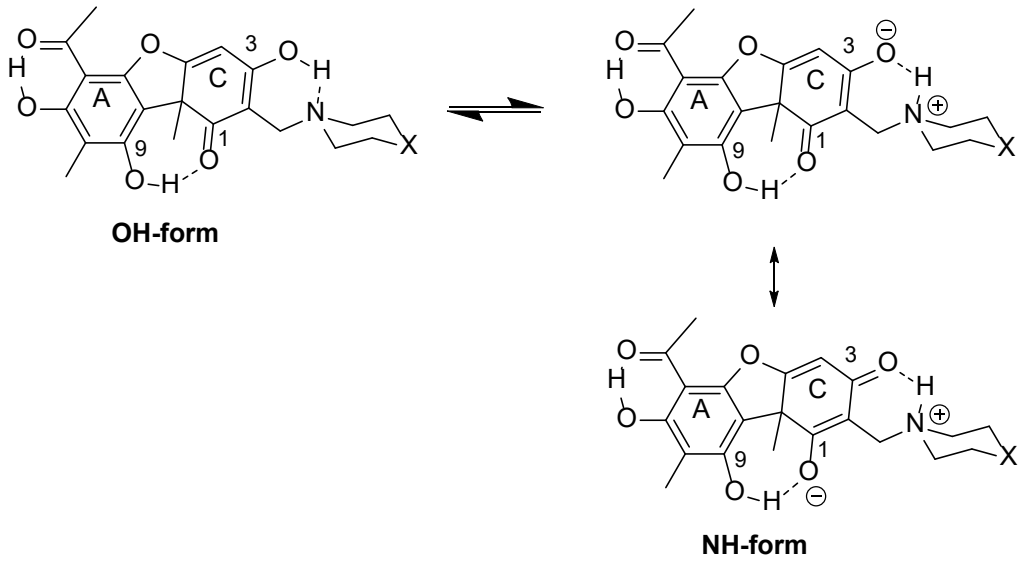
b. Calculated in the PCM approximation using DMSO as solvent.

Discussion

The deacylation reaction resulted in new Mannich bases as shown in Scheme 1 with interesting NMR properties and attack at C-2. However, the loss of an acetyl group is highly unprecedented. The target could have been an enolizable $C{=}OCH_3$ group. However, in the present case the attack occurred at C-2 leading to a loss of a CH_3CO group (For a suggested reaction mechanism see Suppl. Mat). In the case of **5** the acetyl group turns up as N-acetyl-N-methylpiperazine.

The Mannich bases as seen in Schemes 1 and 3 can form a hydrogen bond but a proton transfer is clearly also a possibility. The OH chemical shift of XH-3 is intermediate, ~ 9 ppm (See Table 5). The solvent plays a role as seen especially for the OH-9 hydrogens. As judged both from the OH chemical shifts and from the two-bond deuterium isotope effects on chemical shifts at C-9 the hydrogen bond is stronger in the present compounds than in usnic acid itself. The solvent also influences this hydrogen bond as seen from the difference in $CDCl_3$ and DMSO-d_6 (Table 5). Tautomerism could also be at play as often seen in Mannich bases [17,18], see Scheme 3. From Table 1 it is seen that the ^{13}C chemical shifts are different in $CDCl_3$ and in DMSO-d_6 with differences: for C-1 2.42 ppm; C-2 1.13 ppm; C-3 1.45 ppm; C-4a 1.7 ppm; CH_2 3.92 ppm. This again clearly indicates that the attachment is at the C-ring but also that tautomerism most likely is at play. The latter is indicated as the calculated differences in nuclear shieldings (OH-form – NH-form) as seen in Table 4 are large for the carbons mentioned above C-1 -6.6 ppm; C-2 -8.7 ppm; C-3 8.8 ppm, C-4 6.9 ppm and CH_2 3.7 ppm.

The deuterium isotope effect on chemical shift observed at C-3 in $CDCl_3$ is much smaller than expected if the OH form of Scheme 3 was dominant. Two-bond isotope effect of this kind would typically be large, 0.75 ppm, as judged from the graph of Fig. 6 of the published data [19]. This isotope effect would then gradually become smaller and turn negative as the equilibrium is shifted towards the NH-form [20]. In DMSO-d_6 the effect is -0.13 ppm clearly showing that the tautomeric equilibrium has shifted even further towards the NH-form. A very interesting consequence is the much stronger hydrogen bond between OH-9 and C-1 (C=O) as seen from the large two-bond isotope effect at C-9 in DMSO-d_6 (see Scheme 2) as well as the high OH chemical shift (Table 4). It is seen from a comparison of the OH-7 chemical shift, that this value is very similar in $CDCl_3$ and in DMSO-d_6 (Table 5), which means that DMSO cannot perturb a strong hydrogen bond. The fact that the OH-9 chemical shift is much higher in DMSO-d_6 and that the two-bond deuterium isotope effect on chemical shifts is much higher must be due to the change in the tautomeric equilibrium involving the XH-3 Such a trend is seen for all five compounds.



Scheme 3. Tautomerism and resonance forms of the Mannich base

Table 5. XH chemical shifts of Mannich bases

Com-pound/ XH	Morpho line $CDCl_3$	Morpho line DMSO-d_6	N-Methylpiper azine $CDCl_3$	N-Methylpiper azine DMSO-d_6	3-Piperidi nol DMSO -d_6	4-Piperidi nol DMSO -d_6	Piperid ine $CDCl_3$	Piperid ine DMSO -d_6
OH-7	13.31	13.41	13.29	13.42	13.48	13.39	13.38	13.41
OH-9	13.11	14.98	13.34	15.01	14.98 15.11*	15.01	13.31	15.06
XH-3	missing	8.93	Missing	8.67	8.62	8.42	9.7	8.39

Having established that the OH-3 is hydrogen bonded means that the asymmetric carbon C-9b makes the chemical shifts of C-2′and C-6′ different (see Tables 1-3). However, in compound **4** for C-4′ two sets of signals are seen. C-2′, C-6′and C-3′,C-5′also show differences within the pair (Table 3). If we look at hydroxycyclohexane the largest chemical shift differences for the equatorial and the axial form are seen for C-1, followed by C-3 and C-2. The large differences in chemical shifts for the two resonances of C-4′, C-2′and C-3′for **4** can then be explained by assuming that the OH substituent can both be equatorial and axial. For this to be possible the formaldehyde must have attacked the 4-hydroxypiperidine equally well at the axial and the equatorial position followed by a flip of the ring to have the CH_2R group is in the equatorial positon in both cases.

Conclusion

The synthesized new Mannich bases show strong intramolecular hydrogen bonding. The use of OH chemical shifts and deuterium isotope effects on ^{13}C chemical shifts allows to estimate the hydrogen bond strength. Calculation of ^{13}C chemical shifts for the tautomers and deuterium isotope effects on ^{13}C chemical shifts are essential in order to establish that tautomerism is occurring.

Experimental

NMR

The NMR spectra were recorded on a Bruker 400 Nanobay in either $CDCl_3$ or DMSO-d_6 using TMS as reference. DEPT and HMBC spectra were recorded according to normal procedures. Deuteriation was achieved by dissolving the compounds in CH_3OD and evaporating off the methanol on a rotary evaporator.

Compounds

(+)-6-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-piperidin-1-ylmethyl)dibenzo[b,d]furan-1-9bH)-one (**1**). (+)-6-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-morpholine-1-ylmethyl)dibenzo[b,d]furan-1-9bH)-one (**2**). (+)-6-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-3´-hydroxypiperidin-1-ylmethyl)dibenzo[b,d]furan-1-9bH)-one (**3**). (+)-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-4´-hydroxypiperidin-1-ylmethyl)dibenzo[b,d]furan-1-9bH)-one (**4**). (+)-6-Acetyl-3,7,9-trihydroxy-8,9b-dimethyl-2-N-methylpiperazine-1-ylmethyl)dibenzo[b,d]furan-1-9bH)-one (**5**). For a description of the synthesis see Supp. Mat.

Theoretical calculations

The molecular geometries were optimised using the Gaussian09 suite of programs [21]. Density Functional Theory (DFT) (Beckes [22] exchange and Lee, Yang, Parr [23] correlation term, B3LYP and basis set 6-31G (d,p) was used. The nuclear shieldings were calculated using the GIAO approach [24,25].

Supplementary material

The supplementary material shows 1D ^{1}H and ^{13}C NMR spectra of compounds **1**-**5** and HMBC spectra of **1** - **5**, synthetic procedures and a suggested reaction scheme for the Mannich reaction.

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