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Determinations of the Configuration of C-20 in Derivatives of Adynerin Using DFT/HF Methods*

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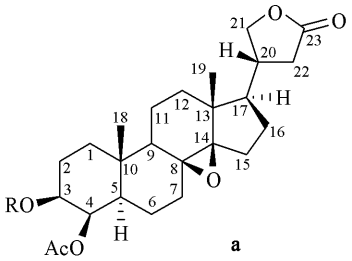
The configurations of C-20 in derivatives of novel 5α-adynerin type, co-existing glycoside in pair, were identified with the calculated chemical shifts of carbon at the B3LYP/6-311 + G(2*d*, *p*) level. These glycosides are unusual cardic aglycones without the common olefin bond in ring E.

Keywords Configuration determination; Adynerin derivative; B3LYP; GIAO; NMR computation

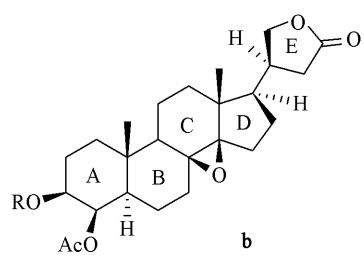
Introduction

Configuration determinations of novel complex natural products are one of the important challenges in stereochemistry. X-ray crystallography[1], circular dichroism[1], NMR analysis of Mosher esters[2,3], and magnetic optical rotation *etc.*[4], are widely used methods for configuration identifications. However, these methods have their own drawbacks. For example, X-ray requires crystal preparation. Mosher ester synthesis needs a definite quantity of natural compound. Traditional 2D NMR spectra sometimes cannot give clear enough correlation relationships between H—H or H—C atoms because of serious resonance overlap of some ^{1}H signals. Recently, computational methods for atomic chemical shift calculations have been developed, including GIAO[5—9], CSGT and LORG[5—9] using HF, DFT or MP2 method[10—12].

The computations of ^{13}C NMR spectra have been widely studied among the ^{1}H[13], ^{3}He[14], ^{15}N[13], ^{19}F[15], ^{27}Al[15], ^{29}Si[16], ^{99}Ru[17] and other nucleus isotopes. Up to now, many valuable computational results have been achieved in ^{13}C spectroscopy studies[18—29]. These achievements have greatly encouraged the uses of NMR spectroscopy calculations in the identification of complex natural compounds. Herein lies the determination of configurations of C-20 for 5α-adynerine derivatives, **1a, 1b** to **5a, 5b** (Scheme 1) through ^{13}C NMR spectra computed by means of the GIAO method at the B3LYP/6-311 + G(2*d*, *p*) level and application of these computed carbon chemical shifts.



a



b

Scheme 1 Structures of five pairs of co-existing isomers

1a, 1b: R = H; **2a, 2b**: R = α-L-cymaropyranosyl; **3a, 3b**: R = β-D-glucopyranosyl(1 - >4)-α-L-cymaropyranosyl;
4a, 4b: R = β-D-glucopyranosyl(1 - >6)-β-D-glucopyranosyl(1 - >4)-α-L-cymaropyranosyl;
5a, 5b: R = β-D-glucopyranosyl(1 - >6)-β-D-glucopyranosyl(1 - >6)-β-D-glucopyranosyl(1 - >4)-α-L-cymaropyranosyl

Molecules of compounds **1a** and **1b** have more than one stereogenic centers but differ in configuration because of only one center at C-20 in ring E. Thus, a different carbon near C-20 in compound **1a** could have a different chemical shift from that of the corresponding carbon in compound **1b**. Therefore, chemical shift differences between the carbons near C-20 of compounds **1a** and **1b** would be specific values. If the

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chemical shift values for compounds **1a** and **1b** and the chemical shift differences between those carbons could be accurately computed, the configuration at C-20 could be established by comparing the magnitude of the computed chemical shifts and their differences with the experimental values.

The mixtures of compounds **a** and **b,** and of compounds **3**, **4** and **5** from *Parepigynum funingense* were reported[30]. To exclude that the mixtures of **a** and **b** were atropisomers in solution in this study, the partial PES computations were done first at the HF/6—31G (*d*) level of theory when the single bond C17-C20 rotated every 10 degree from 0 to 360 degree. The computation results confirmed that the isomers **a** and **b** are not atropisomers. However, the absolute configurations at C-20 in compounds **a** and **b** were not identified for compounds **3**, **4** and **5** in that study. Recently, the mixtures of compounds **1a,** and **1b,** and compounds **2a,** and **2b** were, respectively, obtained again. The **a** and **b** mixtures of compound **3** were separated from each other successfully. Cardenolides can be used as antitumor reagents[31—33] and for treatment of congestive heart failure[34, 35]. The derivatives of 5α-adynerin, a major type in cardenolides, have only been found in *Nerium odorum*[36] and no bioactivity studies reported. Correlations of H—H, H—C in 2D NMR spectra cannot provide clear evidence to assign the configuration at C-20 because of the resonance overlap of some ^{1}H NMR peaks. Moreover, no crystals have been obtained. Thus, the determination of the configuration at the C-20 stereogenic center in these pairs of compounds becomes a challenge.

Computational Method

In our previous study[37], the HF/3-21G* (Gaussian 03) level of theory was used to search for the lowest energy conformations of chiral ligands derived from natural alkaloid *abrine*, Lancifodilactone G, the precursors of transition states in sodium borohydride reductions, and ^{13}C NMR computations[38—42]. Herein, this method was also used to obtain the lowest energy conformations of each isomer of compounds **1a** and **1b**. Partial PES analysis was conducted to see whether compounds **a** and **b** were atropisomers or not at the HF/6-31G (*d*) level when the single bonds of C-17 and C-20 rotated every 10 ° from 0 ° to 350 °[30]. These lowest energy conformations were then further optimized at the HF/6-31G (*d*), B3LYP/6-31G (*d*) and B3LYP/6-31 + G(*d, p*) levels of theory, respectively. Four methods, methods A to D, were used to compute the NMR chemical shifts of ^{13}C. Method A: the NMR data were obtained *via* the B3LYP/6-311 + G(2*d, p*) level of theory on the basis of the B3LYP/6-31 + G(*d, p*)-optimized geometries [B3LYP/6-311 + G(2*d, p*) // B3LYP/6-31 + G(*d, p*)]. Method B: the NMR values were calculated *via* B3LYP/6-311 + G (2*d, p*) // B3LYP/6-31G(*d*). Method C: the NMR magnitudes were computed *via* B3LYP/6-311 + G(2*d, p*) //HF/6-31G(*d*). Method D: the NMR chemical shifts were calculated *via* HF/6-31G (*d*) //HF/6-31G (*d*). The differences in chemical shifts were obtained by subtracting the ^{13}C chemical shift of compound **1b** from the corresponding chemical shift in compound **1a**. After these calculations, the slope and intercept of the least-squares correlation line were used to scale GIAO isotopic absolute shieldings to obtain the new predicted chemical shifts. These computed chemical shift differences were then compared, respectively, with those from experimental ^{13}C NMR data to determine the C-20 configuration in compounds **1**—**3**.

Results and Discussion

The dried root fraction of *Parepigynum funingense* was extracted with 75% aqueous ethanol three times under reflux. The mixtures of compounds **1a** and **1b,** and compounds **2a** and **2b** were obtained from the ethanol extraction by flash column chromatography on silica gel. Pure compounds **3a** and **3b** were obtained from the extraction. The ^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2, respectively. Determining the absolute configuration at C-20 in compounds **a** and **b** has not been achieved because the key evidence from the correlations of H-21 (protons on C-21), H-22 with H-12, H-16 and H-18 and others in ROESY spectra are not clear. Also, the chemical shifts of H_{α}-12 (δ 0.86 in **a**) or H_{β}-12 (δ 0.91 in **a**) and H-18 (δ 0.90 in **a**) have very similar magnitudes in 2D NMR spectra. All of these problems prevented the identification of which isomer, **a** or **b,** had the *R* configuration at C-20 and which had the *S* configuration. Thus, the computations of ^{13}C NMR spectra were carried out with compounds **1a** and **1b** as the representatives to examine the pertinent chemical shift differences. The B3LYP/6-31 + G(*d, p*), B3LYP/6-31G(*d*) and HF/6-31G (*d*)-optimized structures were selected for the computations of ^{13}C NMR through GIAO method at the B3LYP/6-311 + G(2*d, p*) level, respectively[5—9]. HF/6-31G(*d*) theory (method D) was also used to compute the ^{13}C NMR spectra. In the light of the convenience of reading for experimental researchers, all of the magnetic shielding values for compounds **1a** and **1b** were converted into chemical shifts in which TMS was

used as the inner standard. These calculated chemical shift values are summarized in Table 3.

Table 1 ^{13}C chemical shifts(δ) for all carbons in compounds 1—3

C	1a/1b	2a/2b	3a	3b	C	1a/1b	2a/2b	3a	3b
C-1	37.3	37.0	37.1	37.1	C-21	72.4/72.8	72.4/72.7	72.5	72.8
C-2	27.0	25.0	25.1	25.1	C-22	34.2/34.1	34.1	34.2	34.1
C-3	70.8	75.2	75.3	75.3	C-23	176.7/177.3	176.7/177.2	176.9	177.2
C-4	76.3	72.4	72.4	72.4	CH_3CO—	171.0	170.6	170.8	170.7
C-5	47.8	47.3	47.1	47.1	CH_3CO—	21.1	21.0	21.1	21.0
C-6	24.1	23.9	23.9	24.0	Cymarosyl				
C-7	32.3	32.3	32.3	32.3	C-1′		94.9	94.9	94.9
C-8	64.2	64.2	64.2	64.2	C-2′		31.8	31.7	31.8
C-9	51.2	51.2	51.1	51.2	C-3′		73.3	73.2	73.3
C-10	37.5	37.5	37.5	37.6	C-4′		76.3	78.4	78.4
C-11	16.2	16.2	16.2	16.2	C-5′		66.1	64.9	65.0
C-12	37.3/37.7	37.3/37.7	37.3	37.7	C-6′		18.6	18.5	18.4
C-13	40.9/41.1	40.9/41.1	40.9	41.2	OMe-3′		56.3	56.4	56.3
C-14	70.8/70.7	70.8/70.7	70.8	70.8	Glucosyl				
C-15	27.6	27.5	27.6	27.6	C-1″			101.9	102.1
C-16	26.9/25.9	26.9/25.8	27.0	25.9	C-2″			75.5	75.5
C-17	54.7	54.7	54.7	54.8	C-3″			78.6	78.6
C-18	15.8/15.9	15.7/15.8	15.8	15.9	C-4″			71.8	71.9
C-19	15.3	15.1	15.2	15.2	C-5″			78.7	78.8
C-20	38.0/37.6	38.0/37.6	38.1	37.6	C-6″			63.0	63.1

Table 2 ^{1}H NMR data(500 MHz) of compounds 1—3

	1a/1b[a]	2a/2b[b]	3a[b]	3b[b]
H(C-3)	3.89(m)	3.78(m)	3.77(m)	3.77(m)
H(C-4)	5.60(br, s)	5.47(br, s)	5.44(br, s)	5.44(br, s)
H(C-5)	1.45[c]	1.27[c]	1.26[c]	1.26[c]
H_α(C-12)	0.86[c]/0.91[c]	0.86[c]/0.92[c]	0.86[c]	0.92[c]
H_β(C-12)	1.29[c]/1.40[c]	1.31[c]/1.42[c]	1.31[c]	1.41[c]
H_α(C-16)	1.83[c]	1.82[c]	1.83[c]	1.83[c]
H_β(C-16)	1.42[c]/1.36[c]	1.41[c]/1.36[c]	1.42[c]	1.36[c]
H(C-17)	1.36(m)	1.34(m)	1.34(m)	1.34(m)
Me(18)	0.90(s)	0.89(s)	0.89(s)	0.89(s)
Me(19)	1.27(s)	1.23(s)	1.22(s)	1.22(s)
H(C-20)	2.43/2.41(m)	2.43/2.41(m)	2.43(m)	2.41(m)
H_α(C-21)	3.89/3.84(t, J=8.9)	3.88/3.81(t, J=9.0)	3.88(t, J=9.2)	3.81(t, J=9.2)
H_β(C-21)	4.44/4.33(t, J=8.1)	4.43/4.33(t, J=8.3)	4.43(t, J=8.0)	4.33(t, J=8.0)
H_α(C-22)	2.53/2.65(dd, J=16.2, 7.7)	2.53/2.64(dd, J=16.2, 7.5)	2.53(dd, J=16.2, 7.7)	2.65(dd, J=16.3, 7.5)
H_β(C-22)	2.29[c]	2.28[c]	2.29[c]	2.29[c]
CH_3CO—	2.03(s)	2.10(s)	2.07(s)	2.07(s)
H(C-1′)		5.17(br, s)	5.16(br, s)	5.17(br, s)
H(C-6′)		1.52(d, J=6.3)	1.45(d, J=6.4)	1.45(d, J=6.5)
OMe(3′)		3.37(s)	3.40(s)	3.43(s)
H(C-1″)			5.01(d, J=7.5)	4.98(d, J=7.6)
H_α(C-6″)			4.36(dd, J=12.0, 5.0)	4.38(dd, J=11.5, 5.2)
H_β(C-6″)			4.55(dd, J=12.0, 2.0)	4.56(dd, J=11.5, 2.2)

a. In $CDCl_3$; *b*. in C_5D_5N; *c*. overlapping with other signals.

Table 3 Calculated carbon chemical shift values using four methods and the experimental magnitudes

C	Calculated δ values for **1a** and **1b** ($\delta_{cal,\ 1a}$/$\delta_{cal,\ 1b}$)				Experimental $\delta_{exp,\ 1a}$/$\delta_{exp,\ 1b}$
	Method A[a]	Method B[a]	Method C[a]	Method D[a]	
C-1	41.8/41.2	41.8/41.2	39.7/39.7	30.5/30.5	37.3/37.3
C-2	31.4/31.4	31.5/31.6	29.1/28.9	23.4/23.4	27.0/27.0
C-3	76.4/76.4	75.6/75.6	**72.0/71.9**[b]	59.6/59.6	70.8/70.8
C-4	81.4/81.5	80.8/80.9	**75.9/76.1**	65.3/65.3	76.3/76.3

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C	Calculated δ values for **1a** and **1b** ($\delta_{cal,\ 1a}$ /$\delta_{cal,\ 1b}$)				Experimental $\delta_{exp,\ 1a}$ /$\delta_{exp,\ 1b}$
	Method A[a]	Method B[a]	Method C[a]	Method D[a]	
C-5	55.8/56.7	55.8/56.7	53.2/53.3	39.1/39.1	47.8/47.8
C-6	27.4/27.8	27.4/27.9	**24.9/25.1**	19.6/19.6	24.1/24.1
C-7	36.8/37.0	36.9/37.1	34.8/34.8	28.0/28.0	32.3/32.3
C-8	69.7/69.8	69.2/69.6	**63.6/63.7**	49.9/49.9	64.2/64.2
C-9	57.0/57.0	56.5/56.8	54.5/54.6	41.6/41.6	51.2/51.2
C-10	44.3/44.9	43.9/44.9	42.1/42.5	29.2/29.2	37.5/37.5
C-11	20.2/19.9	20.1/20.0	**17.8/17.8**	13.9/13.9	16.2/16.2
C-12	41.5/41.6	41.6/41.7	**38.8/39.1**	31.0/31.2	37.3/37.7
C-13	48.7/48.8	48.4/48.6	45.9/46.3	33.3/33.6	40.9/41.1
C-14	76.7/76.6	76.3/76.3	**70.4/70.2**	56.0/55.9	70.8/70.7
C-15	32.6/32.7	32.4/32.8	30.3/30.3	24.0/24.0	27.6/27.6
C-16	32.0/30.3	31.7/30.4	29.6/28.1	23.8/22.6	26.9/25.9
C-17	61.3/62.0	61.8/61.9	59.0/58.8	45.4/45.1	54.7/54.7
C-18	15.6/15.8	15.9/15.9	13.4/13.3	13.1/13.1	15.8/15.9
C-19	16.1/16.2	16.3/16.4	13.5/13.7	14.0/14.1	15.3/15.3
C-20	45.4/44.2	44.9/44.3	43.5/42.4	31.5/30.9	38.0/37.6
C-21	75.5/76.4	75.2/75.8	70.6/71.6	60.5/61.2	72.4/72.8
C-22	37.9/37.5	37.8/37.5	**35.5/34.8**	29.6/29.0	34.2/34.1
C-23	181.7/182.3	180.8/181.7	173.0/173.7	162.7/163.3	176.7/177.3
$CH_3C{=}O$	178.8/178.8	178.2/178.4	**170.1/170.1**	160.7/160.7	171.0/171.0
$\mathbf{CH}_3C{=}O$	21.7/21.7	21.7/21.9	**19.6/19.7**	20.0/20.0	21.1/21.1

a. Method A: the NMR data were obtained at the B3LYP/6-311 + G(2*d*, *p*) level of theory on the basis of the B3LYP/6-31 + G(*d*, *p*)-optimized geometries[B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31 + G(*d*, *p*)]; method B: B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31G(*d*); method C: B3LYP/6-311 + G(2*d*, *p*) //HF/6-31G(*d*); method D: HF/6-31G(*d*) //HF/6-31G(*d*). *b*. The bold data are located in the window of δ_{exp} ±2.0.

The trends of the computed chemical shifts for compounds **1a** and **1b** were similar with those observed in the experiment *via* all the four methods. However, the maximum errors by methods A, B and D were very high (*e. g.*, δ 8.0—8.7 of C-5 in Table 3 using four methods). Only method C produced the minimum errors among the four methods. Thus, an empirical method reported by Fosyth *et al* [43,44] was used to correct the chemical shifts. The slope and intercept of the least-squares correlation line were used to scale GIAO isotopic absolute shieldings to obtain the new predicted chemical shifts. The new data are summarized in Table 4. After the corrections, methods A and B have almost the same prediction accuracy, the numbers of the chemical shifts whose magnitudes were in the window of δ_{exp} ±2.0 were 16 (67%). There were 56% of the data located in the window of δ_{exp} ±2.0 using method C. Method D gave the poorest prediction even if the data were corrected, only 40% of the data were located in the range of δ_{exp} ±2.0. The maximum error decreased from δ 8.7 to 4.1 by method A.

Table 4 Corrected chemical shifts for compounds 1a and 1b with slope and intercept of least-squares correlation line

C	Calculated δ values for compounds **1a** and **1b** ($\delta_{cal,\ 1a}$ /$\delta_{cal,\ 1b}$)				Experimental $\delta_{exp,\ 1a}$ /$\delta_{exp,\ 1b}$
	Method A[a]	Method B[a]	Method C[a]	Method D[a]	
C-1	**37.3/36.7**[b]	**37.4/36.8**[b]	**38.0/38.0**[b]	36.8/36.8	37.3/37.3
C-2	**27.2/27.2**	**27.3/27.2**	**27 3/27.0**	29.2/29.2	27.0/27.0
C-3	**71.1/71.1**	**70.7/70.7**	**70.9/70.8**	**70.0/70.0**	70.8/70.8
C-4	**76.0/76.1**	**75.8/75.9**	**75.0/75.2**	74.1/74.1	76.3/76.3
C-5	51.0/51.9	51.2/51.1	51.8/51.9	**46.0/46.0**	47.8/47.8
C-6	**23.3/23.7**	**23.3/23.8**	**22.9/23.1**	**25.1/25.1**	24.1/24.1
C-7	**32.4/32.6**	**32.6/32.8**	**33.0/33.0**	**34.1/34.1**	32.3/32.3
C-8	**64.6/64.7**	**64.4/64.7**	**62.4/62.5**	57.6/57.6	64.2/64.2
C-9	**52.2/52.2**	**51.9/52.2**	53.0/53.1	48.7/48.7	51.2/51.2
C-10	39.8/40.4	**39.5** /40.5	40.5/40.9	35.4/35.4	37.5/37.5
C-11	**16.2/15.1**	**16.1/16.0**	**15.7/15.7**	19.0/19.0	16.2/16.2
C-12	**37.0/37.1**	**37.3/37.3**	**37.1/37.4**	**37.3/37.5**	37.3/37.7
C-13	44.1/44.2	43.9/44.1	44.4/44.8	**39.8/40.1**	40.9/41.1
C-14	**71.4/71.3**	**71.4/71.4**	**69.4/69.2**	64.1/64.0	70.8/70.7
C-15	**28.4/28.3**	**28.2/28.6**	**28.4/28.4**	29.8/29.8	27.6/27.6

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C	Calculated δ values for compounds **1a** and **1b** ($\delta_{cal,\ 1a}$ / $\delta_{cal,\ 1b}$)				Experimental $\delta_{exp,\ 1a}$ / $\delta_{exp,\ 1b}$
	Method A[a]	Method B[a]	Method C[a]	Method D[a]	
C-16	**27.8/26.1**	**27.5/26.2**	**27.7/26.2**	29.6/28.3	26.9/25.9
C-17	**56.4/57.1**	57.1/57.2	57.7/57.5	**52.8/52.4**	54.7/54.7
C-18	11.7/11.9	12.0/12.0	11.2/11.1	18.2/18.2	15.8/15.9
C-19	12.2/12.3	12.4/12.5	11.3/11.5	19.1/19.2	15.3/15.3
C-20	40.9/39.7	40.5/39.9	41.9/40.8	**37.9/37.2**	38.0/37.6
C-21	70.3/71.1	70.3/**70.9**	69.6/70.6	68.9/69.7	72.4/72.8
C-22	**33.5/33.0**	**33.5/33.2**	**33.7/33.0**	**35.2/35.2**	34.2/34.1
C-23	174.0/174.6	174.0/174.9	174.1/174.8	**178.4/179.0**	176.7/177.3
$CH_3\mathbf{C}{=}O$	**171.2/171.2**	**171.5/171.7**	**171.2/171.2**	176.2/176.2	171.0/171.0
$\mathbf{CH}_3C{=}O$	17.7/17.7	17.7/17.9	17.5/17.6	25.5/25.5	21.1/21.1

a. Method A: the NMR data were obtained at the B3LYP/6-311 + G(2*d*, *p*) level of theory on the basis of the B3LYP/6-31 + G(*d*, *p*)-optimized geometries[B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31 + G(*d*, *p*)]; method B: B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31G(*d*); method C: B3LYP/6-311 + G(2*d*, *p*) //HF/6-31G(*d*); method D: HF/6-31G(*d*) //HF/6-31G(*d*). *b*. The bold data are located in the window of $\delta_{exp} \pm 2.0$.

Because compounds **1a** and **1b** were a pair of isomers, the chemical shift difference, $\Delta\delta_{cal}$, was established by subtracting the specific chemical shift in compound **1b** from the corresponding value in compound **1a**. Owing to the fact that the systematic errors were removed on taking these differences, the computed $\Delta\delta_{cal}$ magnitudes could be compared with those obtained from the measured chemical shift difference $\Delta\delta_{exp}$ obtained from the ^{13}C NMR spectra. The magnitudes of these chemical shift differences are listed in Table 5. The experimental chemical shift differences of ^{13}C for compounds **1** to **3** are also listed in Table 5.

Table 5 Chemical shift differences of the selected carbons in compounds 1 to 3[a]

C in **a** and **b**	Calculated $\Delta\delta_{cal}$/Corrected $\Delta\delta_{cal}$ for (**1a**—**1b**)[b]				Experimental $\Delta\delta_{exp}$[a]		
	Method A	Method B	Method C	Method D	**1a**—**1b**	**2a**—**2b**	**3a**—**3b**
C-12	-0.1/-0.1	+0.1/0	-0.3/-0.3	-0.2/-0.2	-0.4	-0.4	-0.4
C-13	-0.1/-0.1	-0.2/-0.2	-0.4/-0.4	-0.3/-0.3	-0.2	-0.2	-0.3
C-14	+0.1/+0.1	0/0	+0.2/+0.2	+0.1/+0.1	+0.1	+0.1	0.0
C-16	+1.7/+1.7	+1.3/+1.3	+1.5/+1.5	+1.2/+1.3	+1.0	+1.1	+1.1
C-17	-0.7/-0.7	-0.1/+0.6	+0.2/+0.2	+0.3/+0.4	0.0	0.0	-0.1
C-20	+0.2/+1.2	+0.6/+1.1	+1.1/+1.1	+0.6/+0.7	+0.4	+0.4	+0.5
C-21	-0.9/-0.8	-0.6/-0.6	-1.0/-1.0	-0.7/-0.8	-0.4	-0.4	-0.3
C-22	+0.4/+0.5	+0.3/+0.3	+0.7/+0.7	+0.6/0	0.0	0.0	+0.1
C-23	-0.6/-0.6	-0.9/-0.9	-0.7/-0.7	-0.7/-0.6	-0.6	-0.5	-0.3

a. The experimental $\Delta\delta_{exp}$ for compounds **4** and **5** are almost the same as the data of **1** to **3** listed here. See Ref. [16] for details. *b*. Here x**a**—x**b** means that differences in chemical shifts were obtained by subtracting the ^{13}C chemical shifts of x**b** from the corresponding chemical shifts in x**a**, x = 1, 2, 3.

On the basis of the comparison of the computed chemical shifts (δ_{cal}) and their differences ($\Delta\delta_{cal}$) with the experimental data together (δ_{exp}, $\Delta\delta_{exp}$), it was found that both of the patterns of δ_{cal} values (Tables 3 and 4) and $\Delta\delta_{cal}$ (Table 5) are almost the same as the experimental ones. Therefore, the configuration at C-20 can be established. It is *S* configuration at C-20 in compound **a** and *R* configuration in compound **b** for all compounds **1** to **3**.

The four methods need different computation time and have different computation accuracies. Method A, B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31 + G(*d*, *p*), is the most expensive computation. Even so, it did not give the nearest predictions of the chemical shifts (δ_{cal}) and the chemical shift differences ($\Delta\delta_{cal}$) to the experimental results (δ_{exp}, $\Delta\delta_{exp}$). In contrast, it produced the largest prediction errors among the four methods. In most cases, it had 4 chemical shifts (δ_{cal}) bigger than the experimental values (Table 3, from C-1 to C-17, and C-20, C-23, C-24). In some cases, the prediction errors increased to about $\delta 8$ (Table 3, C-5 and C-13). Method B, which used B3LYP/6-311 + G(2*d*, *p*) //B3LYP/6-31G(*d*) theory, needed less computation time than method A did. This method also gave big over-estimated chemical shifts as method A predicted (Table 3). However, it gave the good prediction of $\Delta\delta_{cal}$ values (Table 4). Method C needed much less computation time than methods A and B. This method gave very good δ_{cal} magnitude (Table 3). The errors between the calculated δ_{cal} values and experimental δ_{exp} are normally less than $\delta 2.0$ in many cases (see the bold numbers in Table 3). It also provides quite good $\Delta\delta_{cal}$ predictions (Table 4). Among all the four methods, method D needed the shortest computation time and provided almost the same good $\Delta\delta_{cal}$ values as method B or C predicted. Unfortunately, this

method gave very poor values of carbon chemical shift, δ_{cal}. Methods B and D are both reasonable ways for computing the differences of the carbon chemical shifts $\Delta\delta_{cal}$. For example, the computed $\Delta\delta_{cal}$ values of C-16 in compounds **1a** and **1b** are +1.3 and +1.2, respectively, using methods B and D (Table 5, entry 5), the determined value is δ +1.0. However, the prediction accuracy from all the methods could be greatly improved after the slope and intercept of the least-squares correlation line were used to scale GIAO isotopic absolute shieldings to obtain the new predicted chemical shifts. In view of the routine practice use, methods B and C were recommended for the computations of NMR magnetic shielding first and then these data could be corrected by means of slope and intercept of least-squares correlation line.

In summary, four methods were used to calculate the carbon chemical shifts and then to compute the differences of the corresponding ^{13}C NMR between two isomers (*e. g.*, compounds **a** and **b**). The computed ^{13}C chemical shifts and their differences between one pair of isomers can be compared with the experimental data for the determination of the stereogenic center during the identifications of pairs of isomers, especially in the structure study of complex natural products.

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References

[1] Humpf H. U., Berova N., Nakanishi K., *et al*, *J. Org. Chem.*, **1995**, *60*, 3539
[2] Kang F. A., Yin C. L., *Chem. Commun.*, **1997**, 579
[3] Dale J. A., Mosher H. S., *J. Am. Chem. Soc*, **1973**, *95*, 512
[4] Parkinson R. A., Oddershede J., *Int J. Quant Chem.*, **1997**, *64*, 599
[5] London F., *J. Phys Radium, Paris*, **1937**, *8*, 397
[6] McWeeny R., *Phys Rev*, **1962**, *126*, 1028
[7] Ditchfield R., *Mol Phys*, **1974**, *27*, 789
[8] Dodds J. L., Mcweeny R., Sadlej A., *J. Mol Phys*, **1980**, *41*, 1419
[9] Wolinski K, Hilton J. F., Pulay P., *J. Am. Chem. Soc*, **1990**, *112*, 8251
[10] Lee T. J., Scuseria G. E., Ed: Langhoff S. R., *Quantum Mechanical Electronic Structure Calculations with Chemical Accuracy*, Kluwer Academic Press, Dordrecht, **1995**
[11] Helgaker T., Jφrgensen P., Olsen J., *Molecular Electronic Structure Theory*, John Wiley and Sons, New York, **2000**
[12] Helgaker T., Jaszunski M., Ruud K, *Chem. Rev*, **1999**, *99*, 293
[13] Wang G. W., Zhang X. H., Zhan H., *et al*, *J. Org. Chem.*, **2003**, *68*, 6732
[14] Ramalho T. C., Buehl M., *Magn. Reson. Chem.*, **2005**, *43*, 139
[15] Bryant P. L., Harwell C. R., Mrse A. A., *et al*, *J. Am. Chem. Soc*, **2001**, *123*, 12009
[16] Sahai N., Tossell J. A., *Inorg. Chem.*, **2002**, *41*, 748
[17] van Gaemers S., Slageren J., O'Connor C. M., *et al*, *Organometallics*, **1999**, *18*, 5238
[18] Wiberg K. B., Hammer J. D., Zilm K. W., *et al*, *J. Org. Chem.*, **2004**, *69*, 1086
[19] Cheng M., Li Q., Lin B., *et al*, *Tetrahedron: Asymm.*, **2006**, *17*, 179
[20] Sebag A. B., Friel C. J., Hanson R. N., *et al*, *J. Org. Chem.*, **2000**, *65*, 7902
[21] Sebag A. B., Forsyth D. A., Plante M. A., *J. Org. Chem.*, **2001**, *66*, 7967
[22] Kirss R. U., Forsyth D. A., Plante M. A., *J. Org. Chem.*, **2003**, *68*, 206
[23] Rodriquez M., Terracciano S., Cini E., *et al*, *Angew. Chem., Int Ed Engl*, **2006**, *45*, 423
[24] Gill G., Pawar D. M., Noe E. A., *J. Org. Chem.*, **2005**, *70*, 10726
[25] Casarini D., Lunazzi L., Mazzanti A., *J. Org. Chem.*, **1997**, *62*, 7592
[26] Besley N. A., Titman J. J., Wright M. D., *J. Am. Chem. Soc*, **2005**, *127*, 17948
[27] Stahl M., Schopfer U., Frenking G., Hoffmann R. W., *J. Org. Chem.*, **1996**, *61*, 8083
[28] Strohmeier M., Grant D. M., *J. Am. Chem. Soc*, **2004**, *126*, 966
[29] Rickard G. A., Karadakov P. B., Webb G. A., *et al*, *J. Phys Chem. A*, **2003**, *107*, 292
[30] Hua Y., Han L. D., Chen C. X., *Helv. Chim. Acta*, **2004**, *87*, 516
[31] Chang L. C., Gills J. J., Bhat K. P., *et al*, *Bioorg. and Med. Chem. Lett*, **2000**, *10*, 21
[32] Manna S. K, Sah N. K, Newman R. A., *et al*, *Can. Res*, **2000**, *14*, 60
[33] Wang X. M., Plomley J. B., Newman R. A., *et al*, *Anal Chem.*, **2000**, *72*, 3547
[34] Malcolm S. B.; Ed: Rosensal G. A., Bumbaum M., *The Chemical Participants*, Academic Press, San Diego, **1991**, 251
[35] Abbott A. J., Holoubek C. G., Martin R. A., *Biochem. and Biophys Res Commun.*, **1998**, *251*, 256
[36] Abe F., Yamauchi T., *Chem. Pharm. Bull*, **1978**, *26*, 3023
[37] Frisch M. J., Trucks G. W., Schlegel H. B., *et al*, *Gaussian 03 User's Reference*, Gaussian Inc., Carnegie, PA, USA, **2003**
[38] Zhu H. J., Jiang J. X., Saobo S., *et al*, *J. Org. Chem.*, **2005**, *70*, 261
[39] Xiao W. L., Zhu H. J., Shen Y. H., *et al*, *Org. Lett*, **2005**, *7*, 2145
[40] Ren J., Li L. C., Liu J. K, *et al*, *Eur J. Org. Chem.*, **2006**, 1991
[41] Li L. C., Jiang J. X., Ren J., *et al*, *Eur J. Org. Chem.*, **2006**, 1981
[42] Liu D. Z., Wang F., Liao T. G., *et al*, *Org. Lett*, **2006**, *8*(25), 5749
[43] Forsyth D. A., Sebag A. B., *J. Am. Chem. Soc*, **1997**, *119*, 9483
[44] Barone G., Gomez P. L., Duca D., *et al*, *Chem. A: Eur J.*, **2002**, *8*, 3233