Determination of the Absolute Configuration of Chiral Secondary Alcohols from Tetra-*O*-acetylglucosidation-Induced ^{1}H Nuclear Magnetic Resonance Shifts

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Tetra-*O*-acetylglucosidation-induced ^{1}H nuclear magnetic resonance shifts were found highly characteristic of the absolute configuration of representative examples of *R* and *S* chiral secondary alcohols. The most important chemical shift changes of structurally diagnostic value for the determination of the absolute configuration of alcohols concern the protons of the aglycone attached to the carbons at β-position relative to the oxygen atom.

Considerable effort was directed during the recent years toward the invention of efficient methods for the determination of the absolute configuration of chiral secondary alcohols.[1] Based on the conformational properties of the glucosidic linkage[2] (Figure 1) and on characteristic glucosidation-induced carbon-13 NMR chemical shift variations, a new technique was developed in this respect[3] a few years ago by Seo and co-workers. According to this method, the strategy for the determination of the absolute configuration of chiral secondary alcohols consists of the following five steps (carbon-13 NMR spectra were recorded in pyridine-d_5 solution): (1) Measurement of the ^{13}C NMR spectrum of the secondary alcohol. (2) Chemical synthesis of the β-D- or α-D-glucopyranoside of the secondary alcohol. (3) Measurement of the ^{13}C NMR spectrum of either the β-D- or the α-D-glucopyranoside of the alcohol. (4) Taking into account carbon-13 NMR chemical shift values for methyl α-D- or β-D-glucopyranoside, calculation of the glucosidation-induced shift variations for the anomeric carbon of the sugar moiety and for the α- and β-carbons of the aglycone as follows:

$$\Delta\delta(\text{anomeric carbon}) = \delta(\text{alcoholic glucoside}) - \delta(\text{methylglucoside})$$

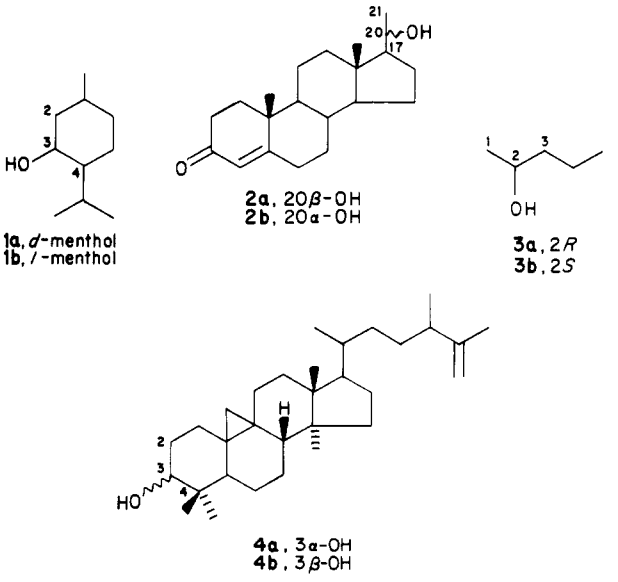
$$\Delta\delta(\alpha \text{ and } \beta \text{ carbons}) = \delta(\text{alcoholic glucoside}) - \delta(\text{alcohol})$$

(5) Determination of the absolute configuration of the secondary alcohol by comparing the calculated ^{13}C NMR shift changes with glucosidation shifts highly characteristic of known alcohols of *R* or *S* absolute configuration and summarized in a table.

From the point of view of the proposed method and the substitution pattern of the neighbors of the oxymethine carbon center, the chiral secondary alcohols were divided into four categories: (a) Two methylene-type carbons are at β-position relative to the oxygen atom of the alcohol. This situation reflects a sterically unhindered case. (b) One or two substituents are located at the syn-β-carbon (relative to the oxygen atom of the pyranose ring) (Figure 1). This situation was named *sterically hindered case I*. (c) One or two substituents are located at the anti-β-carbon (relative to the oxygen atom of the pyranose ring) (Figure 1). This situation was designated *sterically hindered case II*. (d) One or two substituents are located at both the syn- and anti-β-carbons (Figure 1). This case was not investigated.

Results and Discussion

In view of the recent commercial availability of high-field NMR spectrometers we were interested in glucosidation-induced proton chemical shift variations for the determination of the absolute configuration of alcohols. Therefore a 400-MHz ^{1}H NMR spectroscopic investigation was undertaken. Tetra-*O*-acetylglucosidation-induced ^{1}H NMR shifts were calculated as indicated above in connection with the ^{13}C NMR work.[3] $\Delta\delta_S$ = δ (alcoholic glc-Ac$_4$) − δ(methyl glc-Ac$_4$) for the sugar moiety and $\Delta\delta_A$ = δ(alcoholic glc-Ac$_4$) − δ(alcohol) for the aglycone unit. The data we report here on representative examples of alcohols corresponding to hindered cases I and II (**1a,b**, **2a,b**, **3a,b**, and **4a,b**) (Table I) reveal interesting results. Dramatic



1a, *d*-menthol
1b, *l*-menthol

2a, 20β-OH
2b, 20α-OH

3a, 2*R*
3b, 2*S*

4a, 3α-OH
4b, 3β-OH

chemical shift changes of structurally diagnostic value can be detected for the hydrogen attached to the α-carbon and for the protons on the syn- and/or anti-β-carbon of the aglycone. Negative $\Delta\delta_A$ (anti-β-proton) values (−0.02 to −0.27 ppm) were observed for *R*-alcoholic β-D-glc-Ac$_4$ (**5**, **6**, **7**, and **8**) and for *S*-alcoholic α-D-glc-Ac$_4$ (**9**) (sterically hindered case I). On the other hand, positive $\Delta\delta_A$ (syn-

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Table I. ^{1}H NMR Chemical Shifts of the Tetra-*O*-acetylglucosides and Tetra-*O*-acetylglucosidation Shifts (in Parentheses)[a] in $CDCl_3$ for Chiral Secondary Alcohols

			carbohydrate protons[b] ($\Delta\delta_S$), ppm				aglycone protons ($\Delta\delta_A$), ppm		
	glc-Ac_4	alcohol	H-1′	H-2′	H-5′	H-6′ pro-*R*	α-protons	syn-β-protons	anti-β-protons
					Sterically Hindered Case I[c,d]				
5	β-D	(*R*)-**1b**	4.56 (+0.12)	4.94 (−0.05)	3.68 (−0.02)	4.20 (−0.07)	3.40 [H-3] (−0.01)	1.21 [H-4] (+0.10)	0.82, 1.95 [H-2a, H-2e] (−0.14, −0.02)
6	β-D	(*R*)-**2a**	4.57 (+0.13)	4.94 (−0.05)	3.69 (−0.01)	4.21 (−0.06)	3.78 [H-20] (+0.05)	1.42 [H-17] (+0.07)	1.04 [H-21] (−0.21)
7	β-D	(*R*)-**3a**	4.55 (+0.11)	4.96 (−0.03)	3.69 (−0.01)	4.20 (−0.07)	3.77 [H-2] (−0.01)	1.43, 1.39 [H-3, H-3] (−0.03, 0)	1.11 [H-1] (−0.07)
8	β-D	(*R*)-**4a**	4.50 (+0.06)	4.99 (0)	3.64 (−0.06)	4.22 (−0.05)	3.40 [H-3] (−0.09)		1.55, 1.61 [H-2a, H-2e] (−0.08, −0.27)
9	α-D	(*S*)-**1a**	5.25 (+0.29)	4.85 (−0.05)	4.10 (+0.12)	4.26 (0)	3.42 [H-3] (+0.01)	1.31 [H-4] (+0.20)	0.83, 1.04 [H-2a, H-2e] (−0.13, −0.13)
					Sterically Hindered Case II[c,e]				
10	β-D	(*S*)-**1a**	4.57 (+0.13)	5.00 (+0.01)	3.72 (+0.02)	4.24 (−0.03)	3.32 [H-3] (−0.09)	1.09, 2.13 [H-2a, H-2e] (+0.13, +0.16)	1.29 [H-4] (+0.18)
11	β-D	(*S*)-**2b**	4.59 (+0.15)	4.99 (0)	3.70 (0)	4.25 (−0.02)	3.53 [H-20] (−0.08)	1.23 [H-1] (+0.05)	1.51, 1.35 [H-3, H-3] (+0.05, −0.04)
12	β-D	(*S*)-**3b**	4.56 (+0.12)	4.99 (0)	3.70 (0)	4.25 (−0.02)	3.70 [H-2] (−0.08)	1.32 [H-21] (+0.17)	1.53 [H-17] (+0.2)
13	β-D	(*S*)-**4b**	4.56 (+0.12)	5.04 (+0.05)	3.68 (−0.02)	4.24 (−0.03)	3.14 [H-3] (−0.16)	1.70, 1.88 [H-2a, H-2e] (+0.09, +0.13)	
14	α-D	(*R*)-**1b**	5.16 (+0.20)	4.85 (−0.05)	4.20 (+0.22)	4.24 (−0.02)	3.29 [H-3] (−0.12)	1.04, 2.15 [H-2a, H-2e] (+0.08, +0.18)	1.32 [H-4] (+0.21)

[a] Positive and negative shifts designate down and upfield shifts, respectively. Chemical shifts for the original alcohols are obtained by substracting tetra-*O*-acetylglucosidation shifts from the chemical shifts of the corresponding tetra-*O*-acetylglucosides. [b] Glucosidation shifts for protons H-3′, H-4′, and H-6′ pro-*S* are not shown. These $\Delta\delta$ values were similar for both *R* and *S* alcohols. [c] Linear chain *sec*-*R* (**3a**) and -*S* alcoholic glucosides (**3b**) belong respectively to sterically hindered cases I and II. [d] One or two substituents on the syn-β-carbon. [e] One or two substituents on the anti-β-carbon.

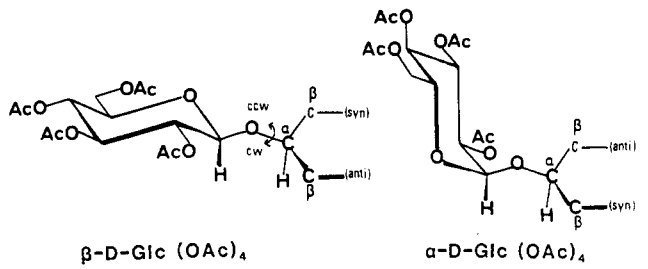

Figure 1.

β-proton) values (+0.05 to +0.18 ppm) were detected for *S*-alcoholic β-D-glc-Ac_4 (**10**, **11**, **12**, and **13**) and for *R*-alcoholic α-D-glc-Ac_4 (**14**) (sterically hindered case II). The α-protons of *R*-alcoholic glc-Ac_4 appeared lower field than those of their *S*-alcoholic glc-Ac_4 counterparts (0.07–0.24 ppm).

Further systematic structurally somewhat less diagnostic chemical shift differences between the tetra-*O*-acetylglucosides of *R* and *S* alcohols are evident from Table I for some carbohydrate protons. Although these differences are smaller than for the α- and β-protons of the aglycones, tetra-*O*-acetyl-β-D-glucosidation shifts for H-2′ and for H-6′ pro-*R* appear characteristic of the absolute configuration of the alcohols. The chemical shift difference for H-2′ and H-6′ pro-*R* between **5** and **10** at 400 MHz was as large as respectively 24 and 16 Hz. These signals resonate at higher field in the *R* with respect to the *S* alcohol derivatives. As far as the two tetra-*O*-acetyl-α-D-glucosides **9** and **14** are concerned, marked differences were detected at 400 MHz for H-1′ (36 Hz) and for H-5′ (40 Hz). The anomeric proton resonates at lower field in the glucoside of the *S* alcohol **9** than in its counterpart **14**, and H-5′ is shielded in the glucoside of the *S* alcohol **9** relative to the glucoside of the *R* alcohol **14**.

All the hydrogen signals of the carbohydrate units and the proton on the α-carbon of the aglycones of the compounds of Table I were resolved and easily identified in $CDCl_3$ solution at 400 MHz. Determination of the chemical shift of the protons of the syn- and anti-β-carbons of the aglycones required spin-decoupling experiments. The hidden β-protons of the triterpenes were revealed by homonuclear shift-correlated (COSY-45) two-dimensional spectra.[4] Chemical shifts for the reference tetra-*O*-acetylmethyl-β-D- and -α-D-glucopyranoside[5,6] were taken respectively for ($CDCl_3$, δ) H-1 (4.44, 4.96), H-2 (4.99, 4.90), H-3 (5.21, 5.49), H-4 (5.09, 5.07), H-5 (3.70, 3.98), H-6 pro-*S* (4.15, 4.10), H-6 pro-*R* (4.27, 4.26).

It appears that the presently proposed ^{1}H NMR method for the determination of the absolute configuration of chiral secondary alcohols is of higher practical value than the technique based on ^{13}C NMR,[3] and the advantage is further substantiated when considering the small amount of substance required for ^{1}H NMR investigations. The most important chemical shift changes of structurally diagnostic value for the determination of the absolute configuration of alcohols concern the protons of the aglycone attached to the carbons at β-position relative to the oxygen atom. The glucosides of Table I were prepared by the Koenigs–Knorr technique.[7]

As a further example, we were interested to check whether the reported β-configuration of liguloxidol (**15**)[8] was correct. In spite of somewhat anomalous results, attributed to the highly crowded environment of the hydroxy group, the ^{13}C NMR method[3] has already confirmed that this sesquiterpenic alcohol belongs to sterically hindered case II. Identical conclusions were reached by ^{1}H NMR spectroscopy. For liguloxidol (**15**) the following proton chemical shifts were measured: δ 2.29 (H-8a), 1.51 (H-8b), 3.66 (H-9), and 1.55 (H-10). As far as the β-D-Glc-Ac_4 of

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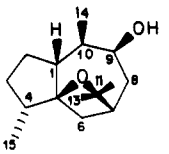

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15 is concerned, it exhibited in its ^1H NMR spectrum the following signals: δ 2.36 (H-8a), 1.63 (H-8b), 3.73 (H-9), and 1.69 (H-10). For the somewhat less diagnostic carbohydrate protons, the following signals appeared: δ 5.00 (H-2′) and 4.25 (H-6′ pro-*R*). From these results, calculation afforded the tetra-*O*-acetyl-β-D-glucosidation shifts: +0.07, +0.12, +0.07, +0.14, +0.01, and −0.02 ppm for respectively H-8a, H-8b, H-9, H-10, H-2′, and H-6′ (pro-*R*). The positive ($\Delta\delta_A$) values for the β-protons and the very small differences ($\Delta\delta_S$) for the carbohydrate protons were indicative of the *S* configuration of the alcohol. However, $\Delta\delta_A$ for the α-proton appeared anomalous in the light of the data of Table I. This result reflects that caution should be exercised when applying the method to alcohols of a highly crowded environment.

The origin of the different ^1H NMR behavior of the syn- or anti-β-carbon-substituted tetra-*O*-acetylglucosylated alcohols is interpreted as resulting from specific conformational changes. In order to avoid steric nonbonded interactions the alcohol moiety rotates along the O–C_α bond, clockwise in the syn-β-carbon-substituted case and counterclockwise in the anti-β-carbon-substituted case.[2,9] It was of interest to examine whether these conformational modifications were reflected by interresidue ^{13}C–^1H and ^{13}C–^{13}C coupling constants (between the aglycone and carbohydrate moieties) of different magnitude for the *R* and *S* alcohols. Although difficult to evaluate from Karplus curves in terms of precise torsional angles, such differences were actually found. As an example we report here interresidue coupling constants for the [1′-^{13}C]-enriched tetra-*O*-acetyl-β-D-glucopyranoside of **4a** and **4b** prepared from [1-^{13}C]-D-glucose. The results are in hertz as follows:

	$^3J_{C\text{-}1',H\text{-}3}$	$^3J_{C\text{-}1',C\text{-}2}$	$^3J_{C\text{-}1',C\text{-}4}$	$^2J_{C\text{-}1',C\text{-}3}$
β-D-glc-Ac_4 of **4a**	3.0	0.5	2.3	1.8
β-D-glc-Ac_4 of **4b**	4.5	1.0	2.5	0.5

In conclusion, the ^1H NMR method proposed here allows a rapid an unambiguous determination of the absolute configuration of a chiral secondary alcohol from a single epimer. Preliminary results indicate[10] that the method can be applied to allylic alcohols as well. However, further investigations are necessary to establish whether the technique proposed is useful in such cases when both β-carbons are substituted.

Experimental Section

General Methods. Melting points were determined with a Büchi apparatus and are uncorrected. A Perkin-Elmer Model 141 MC polarimeter and 1-dm tubes were used for measurement of specific rotations. ^1H NMR spectra were recorded in chloroform solution at 400 MHz. ^{13}C NMR spectra were measured in chloroform-*d* solution at 100.62 MHz. Chemical shifts are given in ppm and tetramethylsilane was the internal standard (δ 0.00). Microanalyses were performed by the Service Central de Microanalyse du CNRS. Silica gel 60-G (Merck) activated at 120 °C was the support for TLC and for column chromatography. The term "standard workup" means that the organic layer was washed with water, dried over Na_2SO_4, and filtered, and the solvent was removed at reduced pressure. Except for **8** and **13**, the tetra-*O*-acetylglucosides of Table I are known compounds.[3]

3-*epi*-Cyclolaudenol (4a). To a solution of cyclolaudenol (**4b**) (150 mg, 0.34 mmol) in dry dichloromethane (5 mL) was added pyridinium chlorochromate (110 mg, 0.51 mmol) and kieselguhr (300 mg), and the mixture was stirred for 15 h at room temperature. After filtration and standard workup the residue was crystallized from methanol giving pure cyclolaudenone (140 mg, 95%). To a solution of cyclolaudenone (120 mg, 0.27 mmol) in a mixture of methanol–*N*,*N*-dimethylformamide (5:1) (6 mL) was added sodium borohydride (40 mg, 1.06 mmol). After 4 h of stirring at room temperature and standard workup, the crude product was purified by preparative TLC using hexane–dichloromethane (1:1) to give **4b** (66 mg, 55%) and **4a** (48 mg, 40%): mp 94–96 °C; $[\alpha]^{22}_D$ +20° (*c* 0.6, chloroform); mass spectrum, *m*/*z* 440 ($M^{+\cdot}$); ^1H NMR (Table I).

Anal. Calcd for $C_{31}H_{52}O$: C, 84.56; H, 11.81. Found: C, 84.50; H, 11.71.

Tetra-*O*-acetyl-β-D-glucoside of Cyclolaudenol (13). To a solution of cyclolaudenol (**4b**) (35 mg, 0.08 mmol) in dry 1,2-dichloroethane (2 mL) were added penta-*O*-acetyl-β-D-glucose (31 mg, 0.08 mmol), powdered molecular sieves 4-Å (150 mg), and trimethylsilyl trifluoromethanesulfonate (10 mg, 0.04 mmol). After 4 h of stirring at room temperature, the mixture was filtered through kieselguhr. Standard workup gave a residue, which was purified by preparative chromatography using the solvent system hexane–ethyl acetate (7:3). Pure **13** (15 mg, 25%) was obtained after crystallization from acetone–ethanol: mp 227 °C; $[\alpha]^{22}_D$ +17° (*c* 0.3, chloroform); mass spectrum, *m*/*z* 770 (M^+); ^1H NMR (Table I).

Anal. Calcd for $C_{45}H_{70}O_{10}$: C, 70.13; H, 9.09. Found: C, 70.27; H, 8.99.

Tetra-*O*-acetyl-β-D-[1-^{13}C]glucoside of Cyclolaudenol ([1′-^{13}C]-13). This compound was prepared in the same manner as **13** by using penta-*O*-acetyl-β-D-[1-^{13}C]glucose.[6]

Tetra-*O*-acetyl-β-D-glucoside of 3-*epi*-Cyclolaudenol (8). To a solution of 3-*epi*-cyclolaudenol (**4a**) (20 mg, 0.45 mmol) in dry 1,2-dichloromethane (2 mL) were added penta-*O*-acetyl-β-D-glucose (20 mg, 0.05 mmol), powdered molecular sieves 4-Å (100 mg), and trimethylsilyl trifluoromethanesulfonate (10 mg, 0.04 mmol). After 5 h of stirring at room temperature, the mixture was filtered through kieselguhr. Standard workup gave a residue, which was purified by preparative chromatography using the solvent system hexane–ethyl acetate (1:1). Pure **8** (8 mg, 20%) was obtained: mp 164–166 °C; $[\alpha]^{22}_D$ −28° (*c*, 0.18, chloroform); mass spectrum, *m*/*z* 770 (M^+); ^1H NMR (Table I).

Anal. Calcd for $C_{45}H_{70}O_{10}$: C, 70.13; H, 9.09. Found: C, 70.00; H, 9.21.

Tetra-*O*-acetyl-β-D-[1-^{13}C]glucoside of 3-*epi*-Cyclolaudenol ([1′-^{13}C]-8). This compound was prepared in the same manner as **8** by using penta-*O*-acetyl-β-D-[1-^{13}C]glucose.[6]

Acknowledgment. We are indebted to Dr. C. Merienne, Orsay University, France, for his invaluable help in the spectral measurements. P.I. thanks FAPESP (Brazil) for a postdoctoral fellowship.

Registry No. **4a**, 90686-45-6; **4b**, 511-61-5; **5**, 56650-22-7; **6**, 67400-14-0; **7**, 67425-08-5; **8**, 98923-03-6; **13**, 98923-01-4; **8** (1′-^{13}C), 98976-52-4; **9**, 67462-25-3; **10**, 67462-26-4; **11**, 67400-15-1; **12**, 67425-10-9; **13** (1′-^{13}C), 98923-02-5; **14**, 67462-23-1; cyclolaudenone, 2315-13-1; penta-*o*-acetyl-β-D-glucopyranose, 604-69-3; penta-*O*-acetyl-β-D-[1-^{13}C]glucopyranose, 40010-53-5.

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