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Discrimination of the two diastereoisomeric glycosides heterodendrin and epi-heterodendrin by the combined use of NOE and molecular mechanics

Peter P. Lankhorst ^{a, *}, Jan W.H. Smeets ^a, Cornelis A.G. Haasnoot ^b

^a Gist-brocades BV, P.O. Box 1, 2600 MA Delft, Netherlands ^b Diosynth BV, P.O. Box 20, 5340 BH Oss, Netherlands

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Abstract

The two glycosides (S)-heterodendrin and (R)-epi-heterodendrin were synthesized in a novel, one-step enzymatic synthesis, and separated by means of column chromatography. The ¹H NMR spectra of the two diastereoisomers differ mainly in the chemical shift of H-2' of the side chain. At first sight the ¹H NMR spectra do not allow a stereospecific assignment. It was found, however, that the NOE between the anomeric proton H-1 and H-2' of the side chain is considerably larger in epi-heterodendrin than in heterodendrin, which indicates on a time-averaged basis a smaller distance between these two protons in epi-heterodendrin. This difference in conformational behaviour is correctly reproduced by molecular mechanics calculations, thereby offering a method for the discrimination of these two glycosides.

Keywords: MM2; Cyanogenic glycoside; 3D structure; Conformational analysis; Glucosidase

1. Introduction

The nuclear Overhauser effect is a well established tool in structural and conformational studies of organic compounds [1]. For instance, applications of the NOE effect for the discrimination of diastereoisomeric compounds are numerous in the case of more or less rigid cyclic compounds [2]. In those cases the presence or absence of an NOE may give information on the relative position (cis or trans) of substituents on the ring. Note,

^{*} Corresponding author.

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however, that such conclusions rely always on some *a priori* knowledge of the conformation of the compound under investigation.

Usually, in the case of diastereoisomeric open-chain compounds, a number of different conformers is present and one cannot say beforehand which conformers are prevalent. Therefore, it is not simple to predict whether or not a certain set of protons will exhibit an NOE. This problem, however, can be tackled by molecular mechanics (MM) calculations, which predict the low-energy conformations and hence interproton distances.

In the case of conformational averaging, as it is anticipated with the compounds investigated here, NOE data should be treated with some caution. It is well known, that in such cases a direct correlation between the experimental NOE and the average interproton distance exists, however, averaging should be carried out over r^{-6} and not over r. The effect of this procedure is that small interproton distances are emphasized [3].

It is the purpose of this study to compare the interproton distances predicted by MM calculations with experimental NOEs of two diastereoisomeric openchain compounds to verify whether or not this procedure can be used to discriminate between the diastereoisomers. It is noted that a more or less analogous approach was adopted successfully by Osawa and co-workers [4], who showed that diasteromeric hexaalditol peracetates can be correctly identified by comparing the experimental vicinal ${}^{1}\text{H}{-}{}^{1}\text{H}$ coupling constants with those predicted by molecular mechanics and the generalized Karplus equation.

The compounds investigated in the present study are two diastereoisomeric cyanogenic glycosides, i.e., heterodendrin and its C-2' epimer epi-heterodendrin (see Fig. 1). Both compounds are β -D-glucopyranosyl derivatives, differing only in the stereochemistry at C-2'. Heterodendrin was first isolated by Hübel and Nahrstedt [5] from *Heterodendron oleafolium*. Later they demonstrated by means of chemical modification and GLC comparison with 2-hydroxy-isovaleric acid of known configuration that heterodendrin has the (S) configuration at C-2' [6]. Epi-heterodendrin was first isolated by Erb et al. from seedlings of barley *Hordeum vulgare* [7]. It was demonstrated that this compound possesses the (R) configuration at C-2', amongst others, from the fact that epi-hetero-dendrin slowly isomerizes to heterodendrin and *vice versa*.

Obtaining heterodendrin and epi-heterodendrin is not an easy task. Isolation procedures from natural products such as seedlings of barley [7,8] are time-consuming, complicated, and give small amounts of product. Also synthetic methods [9] have been attempted for the production of these natural cyanohydrin glycosides. Numerous variations on the Koenigs-Knorr condensation of α -acetobromoglucose with 2-hydroxy-3methylbutyronitrile gave poor yields of the tetraacetates of heterodendrin and epi-heterodendrin. In this paper a novel one-step enzymatic synthesis for heterodendrin and epi-heterodendrin is presented using glucose as an inexpensive reagent.

2. Experimental

(R,S)-2-Hydroxy-3-methylbutyronitrile.—This compound was synthesized according to a literature procedure [8].



Fig. 1. Structure of: 1, heterodendrin; 2, epi-heterodendrin; 3, model compound for heterodendrin; and 4, model compound for epi-heterodendrin.

Heterodendrin and epi-heterodendrin.—A mixture of 1 kg (5.55 mol) of D-(+)-glucose, 400 mL of diluted phosphate buffer (pH 7), 100 g (1.0 mol) of (R,S)-2-hydroxy-3-methylbutyronitrile, and 1 g of β -glucosidase (Sigma No. G-0395, from almonds) was stirred at 50°C for 72 h. The mixture was extracted 5 times with 0.5 L of acetone and the combined extracts were evaporated to give a two-phased residue. The organic layer was extracted with water twice and the combined water layers were evaporated. The residue was extracted several times with 4:1 CH₂Cl₂-CH₃OH. The combined extracts were evaporated to give ca. 5 g of crude product. This was purified by column chromatography over 100 g of silica (eluent 88:10:2 EtOAc-MeOH-water) giving 1.3 g of a mixture of the (S)-heterodendrin and (R)-epi-heterodendrin. The two diastereoisomers were separated by column chromatography (Merck Fertigsäule C, 88:10:2 eluent EtOAc-MeOH-water) yielding 0.57 g of (S)-heterodendrin (R_f 0.30) and 0.49 g of (R)-epi-heterodendrin (R_f 0.22) both as a colorless oil.

The stereochemistry of both isomers was determined by comparing the experimental NMR spectra with data published previously [7].

NMR spectroscopy.—NMR spectra were recorded on a Bruker AM-600 spectrometer operating at a ¹H frequency of 600 MHz. All spectra were recorded on a 5-mm inverse triple resonance probe, equipped with self-shielded gradients. Samples were prepared containing 10 mg of the glycoside in 0.5 mL Me₂SO-d₆ solution. All chemical shifts were expressed relative to Me₄Si, using the central peak of DMSO as an internal standard [$\delta_{H(Me_2SO)} = 2.50$ ppm and $\delta_{C(Me_2SO)} = 39.6$ ppm]. The ¹H and ¹³C NMR spectra were assigned by a combination of two-dimensional NMR techniques. A phase sensitive double-quantum filtered COSY spectrum was obtained according to Marion et

al. [10]. A ¹H detected ¹³C⁻¹H correlation spectrum (HSQC) [11] was recorded, employing pulsed field gradients for coherence selection [12]. The length of the sinusoidal gradient pulses was 1 ms and the gradient strength was $0.5 \text{ T} \cdot \text{m}^{-1}$. 256 spectra of 1K datapoints each were recorded with 8 scans per increment.

A ¹H detected ¹³C⁻¹H correlation spectrum optimized for two- and three-bond coupling constants (HMBC) [13] was also recorded, employing pulsed field gradients [14]. Sinusoidal gradient pulses were employed of 1 ms length and the gradient strength was 0.5 T \cdot m⁻¹. 256 spectra of 1K datapoints each were recorded with 16 scans per increment. A delay of 83 ms was chosen for evolution of long-range coupling constants.

One-dimensional NOE difference spectra were recorded in Me_2SO-d_6 as well as in D_2O solution at ambient temperature using a multiple irradiation scheme [15,16]. Total irradiation times of 0.5, 1, and 3 s were employed.

Molecular mechanics calculations.—All molecular mechanics calculations were carried out on an IBM compatible PC using the MM2 program [17]. Torsional and bending parameters related to the cyano group were taken from the work of Castells et al. [18], as described below.

3. Results and discussion

Synthesis.—Enzymatic transglycosidation reactions for synthesis of heterodendrin and epi-heterodendrin with 1-phenyl- β -D-glucopyranose or 1-(4-nitrophenyl)- β -D-glucopyranose as glucose donor were not successful.

In contrast, the two cyanogenic glycosides could be prepared easily in a one-step enzymatic synthesis by just stirring a mixture of (R,S)-2-hydroxy-3-methylbutyronitrile (isobutyraldehyde cyanohydrin), D-(+)-glucose, and β -glucosidase at 50°C for 3 days. The two diastereoisomers were separated by means of column chromatography.

Although the yield of heterodendrin and epi-heterodendrin is relatively poor, this method is preferable to previously published methods for the following reasons: the synthesis is very simple and the reagents are inexpensive because D-(+)-glucose can be used in the coupling reaction without prior modification.

NMR spectroscopy.—The ¹H NMR spectra of the two glycosidic diasteroisomers in Me_2SO are shown in Fig. 2. It can seen from Fig. 2 that the spectra of the two compounds closely resemble each other. In fact, all protons have nearly identical chemical shifts, except for the H-2' signal at ca. 4.7 ppm in the bottom spectrum of heterodendrin, which is shifted 0.2 ppm upfield in the upper spectrum of epi-heterodendrin. The assignment of these spectra by means of COSY, HSQC, and HMBC is a relatively easy, standard procedure. The ¹H and ¹³C chemical shift data are collected in Table 1. All observed chemical shifts, including the chemical shifts of the above-mentioned signals, are in full agreement with the chemical shift data published earlier by Erb et al. for heterodendrin and epi-heterodendrin [7]. Therefore, it could be deduced mainly from the chemical shift of H-2' that the compound giving rise to the upper spectrum of Fig. 2 must be heterodendrin, and that the compound giving rise to the bottom spectrum of Fig. 2 must be heterodendrin.



Fig. 2. 600-MHz ¹H NMR spectra of heterodendrin (bottom) and epi-heterodendrin (top) in Me₂SO at ambient temperature.

Table 1

¹H and ¹³C NMR chemical shifts of heterodendrin and epi-heterodendrin, measured in Me₂SO. Shifts are expressed relative to Me₄Si, using the signal of Me₂SO as an internal standard (¹H, $\delta_{Me_2SO} = 2.50$; ¹³C, $\delta_{Me_2SO} = 39.6$)

| Atom number | $\delta_{ m H}$ | $\delta_{\rm C}$ | δ _Η | δ _C |
|-----------------|-----------------|------------------|----------------|----------------|
| | Heterodend | rin | Epi-heterod | endrin |
| 1 | 4.32 | 100.9 | 4.29 | 103.4 |
| 2 | 2.98 | 73.2 | 2.99 | 73.4 |
| 3 | 3.18 | 76.6 | 3.12 | 76.6 |
| 4 | 3.02 | 70.1 | 3.08 | 69.9 |
| 5 | 3.16 | 77.4 | 3.15 | 77.1 |
| 6a | 3.68 | 61.3 | 3.67 | 61.1 |
| 6b | 3.42 | | 3.48 | |
| 1′ | | 117.8 | | 118.6 |
| 2' | 4.74 | 70.7 | 4.55 | 72.6 |
| 3' | 2.04 | 31.1 | 2.07 | 31.1 |
| 4' ^a | 1.01 | 17.9 | 1.00 | 17.5 |
| 5' ^a | 0.98 | 17.2 | 0.99 | 17.0 |

^a Assignment may be interchanged.



Fig. 3. NOE difference spectra resulting from irradation (3 s) of the H-1 proton in heterodendrin (bottom) and epi-heterodendrin (top) in Me_2SO at ambient temperature.

At this point it should be realized that the discrimination between the two compounds relies exclusively on previous work including chemical transformations [6], and that there is no straightforward way to discriminate between the two compounds from their NMR spectra alone. In the following an alternative approach will be outlined.

The NOE difference spectra of heterodendrin and its C-2' epimer in Me_2SO-d_6 are shown in Fig. 3. In both spectra the H-1 proton of the glucose moiety was irradiated. When an irradiation time of 3 s is used, the enhancement is about 9% in the case of epi-heterodendrin, approximately twice as much as the 4% measured for heterodendrin. Similar results were obtained when the H-2' protons of the side chains were irradiated. Comparable experiments were carried out with different irradiation times and in D_2O with similar results.

In all experiments the NOE between H-2' and H-1 of epi-heterodendrin is significantly larger than the NOE between the same protons of heterodendrin. This observation may be interpreted as a smaller time-averaged ${}^{1}H{-}^{1}H$ distance between H-1 and H-2' in epi-heterodendrin than in heterodendrin.

Molecular mechanics calculations.—Although the MM2 program does include bending and torsional parameters related to the cyano group, unfortunately the direct application of MM2 to combinations of a cyano group with some other functional groups is not supported. In the present case the torsional constants for Csp³–Osp³–

| Changed constants | MM2 parameters | |
|--|-------------------------------------|--|
| Nsp-Csp | $l_0 = 1.135, \ ks = 18.500$ | |
| $C_{sp} - C_{sp3} - O_{sp3}$ | $\theta_0 = 105.0, k_b = 0.9$ | |
| $C_{sp} - C_{sp3} - C_{sp3} - C_{sp3}$ | $V_1 = 0.2, V_2 = -0.95, V_3 = 0.2$ | |
| $C_{sp} - C_{sp3} - O_{sp3} - LP$ | $V_1 = 1.0, V_2 = -0.2, V_3 = -0.4$ | |
| $C_{sp} - C_{sp3} - O_{sp3} - C_{sp3}$ | $V_1 = 0.0, V_2 = 0.0, V_3 = 0.0$ | |

Table 2 MM2 parameters related to the cyano group which were taken from [18]

 Csp^3-Csp and $Csp-Csp^3-Osp^3-LP$ (lone pair) and the bending constants for Osp^3-Csp^3-Csp were not available. However, recently Castells et al. [18] published a number of the parameters related to the cyano group. Therefore, the previously missing parameters, as well as all other parameters related to the cyano group, were taken from the work of Castells et al. The numerical values for the parameters used in this paper are summarized in Table 2.

The side chain of heterodendrin and epi-heterodendrin have backbones comprising three torsional angles. Therefore, the number of conformers that may theoretically be present due to side-chain rotamers amounts to 27 for each compound. As the glucose moiety of both molecules has three hydroxyl groups with three possible orientations each and a CH_2OH group with 9 possible orientations the total number of conformations one has to deal with is enormous. Although specialized force fields have been published [19] to deal with this problem, here we avoid too lenghty calculations by excluding the hydroxyl groups and the CH_2OH group from the calculations. This procedure seems justified as it is likely that the orientation of the hydroxyl groups does not influence the conformational equilibrium of the side chain. Thus, MM2 calculations were carried out for the two model compounds (depicted in Fig. 1), which differ from the original compounds in the substitution pattern of the tetrahydropyran moiety. In this way the number of possible conformations is restricted to 27 side chain rotamers for each compound. The energy of all 27 conformations was minimized using the MM2 program [17].

In the case of heterodendrin three low-energy conformers emerge, which may be described as -tt, -t+, and -t-. In this notation the first symbol denotes the conformation of the glycosidic bond (O-5-C-1-O-1-C-2'), or torsion angle ϕ , the second symbol denotes the conformation of C-1-O-1-C-2'-C-3', or torsion angle ψ , and the last symbol denotes the conformation of O-1-C-2'-C-3'-C-4', or torsion angle ω . The '-' stands for gauche⁻, the '+' for gauche⁺, and the t for trans. The actual values predicted for these three backbone angles are summarized in Table 3 together with the MM2 energy and the predicted distance between H-1 and H-2'. Apparently, for the glycosidic torsion angle ϕ the gauche⁻ conformation is favoured, and for ψ the trans conformation. The three low-energy conformations differ in the third dihedral angle, ω , but they have approximately the same steric energy, and the H-1-H-2' distance amounts to 2.64-2.68 Å. Table 3 also lists the conformational properties of the three conformers that are next lowest in energy, i.e., +tt, +t, and +t -. The MM2 energy of these conformers is ca. 11 kJ/mol higher than the MM2 energy of the

Table 3

Table 4

Torsion angles, MM2 steric energies, H-1-H-2' distances, and populations of individual conformers (f) predicted for the six lowest energy conformers of the model compound for heterodendrin (3). The torsion angle, ϕ , corresponds to O-5-C-1-O-1-C-2', the torsion angle, ψ , corresponds to C-1-O-1-C-2'-C-3', the torsion angle, ω , corresponds to O-1-C-2'-C-3'-C-4'

| Shorthand notation | ϕ | ψ | ω | E (MM2) (kJ/mol) | H-1–H-2′ (Å) | f^{a} |
|--------------------|--------|-------|---------|------------------|--------------|------------------|
| -tt | -73.6 | 164.2 | - 179.5 | 93.0 | 2.68 | 0.22 |
| -t+ | -73.5 | 163.1 | 58.2 | 90.9 | 2.67 | 0.50 |
| - <i>t</i> - | -73.2 | 161.1 | - 70.5 | 93.0 | 2.64 | 0.22 |
| +tt | 45.4 | 153.6 | - 179.9 | 103.7 | 3.60 | 0.003 |
| + <i>t</i> + | 45.0 | 154.3 | 58.0 | 102.4 | 3.60 | 0.05 |
| + <i>t</i> - | 46.3 | 153.5 | - 70.5 | 103.6 | 3.60 | 0.003 |

^aCalculated, using the Boltzmann distribution: $f_x / f_0 = e^{-\Delta \epsilon / kT}$, where f_0 denotes the mole fraction of the conformer with the lowest energy, and $\Delta \epsilon$ denotes the energy difference between conformer x and conformer 0. $f_0 + f_1 + f_2 + \dots = 1$.

previously mentioned three conformers. Therefore, it is reasonable to assume that only the -t +, -tt, and -t - conformers are present to a significant extent. All other conformers than the listed six conformers have higher energies.

In epi-heterodendrin the situation is slightly different. Here also three low energy conformers are predicted, i.e., -tt, -t+, and -t-, however, a fourth low-energy conformer emerges with a comparable MM2 steric energy, namely -t. The conformational characteristics of these four conformers are summarized in Table 4, along with the properties of the three conformers next lowest in energy. Other conformers than those listed have significantly higher energies and are not further considered here.

Using the Boltzmann distribution the relative population of each calculated conformer can be computed in a straightforward way. Populations of the predicted conformers of the model compound for heterodendrin and of the model compound for epi-heterodendrin are given in the last columns of Tables 3 and 4, respectively.

Finally, the average distance between H-1 and H-2' was calculated using the expression: $r = [\langle r^{-6} \rangle]^{-1/6}$, where $\langle r^{-6} \rangle$ denotes the population weighted average of r^{-6} . In this way an average value of r = 2.69 Å is obtained for heterodendrin and an average value of r = 2.37 Å for epi-heterodendrin, which is in good agreement with the

| | | | - | | | |
|--------------------|--------|---------|---------|------------------|--------------|------|
| Shorthand notation | φ | ψ | ω | E (MM2) (kJ/mol) | H-1–H-2' (Å) | f |
| - tt | -65.6 | - 159.2 | 175.7 | 94.5 | 2.27 | 0.37 |
| -t+ | - 64.9 | - 159.6 | 56.3 | 96.9 | 2.28 | 0.14 |
| - <i>t</i> - | -65.6 | - 159.3 | -53.7 | 96.6 | 2.27 | 0.16 |
| t | -71.2 | - 79.9 | - 179.0 | 95.2 | 2.73 | 0.28 |
| + t + | 65.8 | - 147.4 | 58.1 | 103.2 | 3.57 | 0.01 |
| + <i>t</i> - | 65.6 | - 147.6 | -53.1 | 103.1 | 3.57 | 0.01 |
| + <i>tt</i> | 65.8 | - 148.9 | 176.0 | 101.6 | 3.57 | 0.02 |
| | | | | | | |

Torsion angles, MM2 energies, H-1-H-2' distances, and populations of individual conformers (f) predicted for the lowest energy conformers of the model compound for epi-heterodendrin (4)

| Shorthand notation | φ | ψ | ω | <i>E</i> (MM2) (kJ/mol) | H-1-H-2' (Å) | f |
|--------------------|--------|-------|---------|-------------------------|--------------|------|
| - tt | - 75.5 | 163.2 | - 179.7 | 94.9 | 2.64 | 0.23 |
| -t + | - 75.4 | 161.4 | 57.9 | 92.8 | 2.62 | 0.53 |
| - <i>t</i> - | - 74.7 | 158.4 | -70.7 | 94.8 | 2.58 | 0.24 |

Torsion angles, MM2 energies, H-1-H-2' distances, and populations of individual conformers (f) predicted for model compound for heterodendrin, bearing an OH group on C-2'

observation of a larger NOE between these two protons in epi-heterodendrin than in heterodendrin.

It could be argued that the model compounds chosen for heterodendrin and its epimer introduce a too dramatic simplification of reality. Therefore we introduced a hydroxyl group on C-2 of the glucose moiety, thereby mimicking more closely the situation in the glycosides. The hydrogen of the hydroxyl group was positioned gauche⁺ with respect to C-1. The calculations were repeated for the three low-energy conformers of heterodendrin and the four low-energy conformers of its epimer. The results are shown in Tables 5 and 6, respectively. If the population weighted average of r^{-6} is calculated in the same way as shown above a value of r = 2.45 Å results for epiheterodendrin, and a value of r = 2.61 Å for heterodendrin. It is clear that, although individual angles and also the H-1-H-2' distances differ slightly from the previously calculated values, the same trend is predicted as before, i.e., on a time-average basis the two hydrogen atoms H-1' and H-2 are located more closely together in epi-heterodendrin than they are in heterodendrin.

Finally, the same calculations were carried out without any simplifications at all, i.e., all hydroxyl groups, as well as the CH_2OH side chain were reintroduced. The hydroxyl groups were oriented in such a way that they form a cooperative lattice of hydrogen

Table 6

Table 5

Torsion angles, MM2 energies, H-1-H-2' distances, and populations of individual conformers (f) predicted for a model compound for epi-heterodendrin, bearing an OH group on C-2'

| Shorthand notation | φ | ψ | ω | E (MM2) (kJ/mol) | H-1-H-2' (Å) | f |
|--------------------|--------|---------|-------|------------------|--------------|------|
| -tt | - 65.3 | -156.9 | 175.8 | 95.2 | 2.27 | 0.32 |
| -t+ | -66.3 | -159.2 | 56.2 | 97.2 | 2.27 | 0.14 |
| - <i>t</i> - | -71.0 | - 161.9 | -54.2 | 97.1 | 2.25 | 0.15 |
| t | - 75.0 | - 86.7 | 177.5 | 94.7 | 2.57 | 0.39 |

Table 7

Torsion angles, MM2 energies, H-1-H-2' distances, and populations of individual conformers (f) predicted for heterodendrin

| Shorthand notation | φ | ψ | ω | E (MM2) (kJ/mol) | H-1–H-2′ (Å) | f |
|--------------------|--------|-------|--------|------------------|--------------|------|
| -tt | - 74.9 | 161.5 | -179.3 | 94.1 | 2.64 | 0.24 |
| -t+ | - 74.7 | 162.1 | 60.0 | 92.0 | 2.66 | 0.52 |
| - <i>t</i> - | - 76.9 | 153.9 | - 69.9 | 93.9 | 2.51 | 0.24 |

| for epi-heterodendrin | | | | | | | |
|-----------------------|--------|---------|--------|-------------------------|--------------|------|--|
| Shorthand notation | φ | ψ | ω | <i>E</i> (MM2) (kJ/mol) | H-1–H-2′ (Å) | f | |
| - tt | -68.0 | - 152.9 | 175.9 | 91.8 | 2.24 | 0.40 | |
| - <i>t</i> + | -68.2 | - 152.9 | 59.3 | 94.3 | 2.25 | 0.15 | |
| - <i>t</i> - | - 69.9 | - 153.6 | - 55.1 | 93.6 | 2.23 | 0.19 | |
| t | - 78.8 | - 86.3 | 177.1 | 92.8 | 2.51 | 0.26 | |

Torsion angles, MM2 energies, H-1-H-2' distances, and populations of individual conformers (f) predicted

bonds, and the C-5-C-6 bond was given the gg orientation (O-5-C-5-C-6-O-6 and C-4-C-5-C-6-O-6 gauche). Again, these calculations were only carried out on the 'low-energy conformers' deduced in the previous sections. The results are collected in Table 7 for heterodendrin and in Table 8 for epi-heterodendrin. It can be seen, that individual torsion angles and interproton distances are not significantly different from those predicted for the model compounds, and the average distance between H-1 and H-2' (when averaged over r^{-6}) is predicted to be 2.61 Å in heterodendrin and 2.29 Å in epi-heterodendrin which values agree well with the values predicted for the model compounds.

4. Conclusions

It follows from NOE difference experiments that the time-averaged ${}^{1}H{-}^{1}H$ distance between H-1 and H-2' is significantly smaller in epi-heterodendrin than in heterodendrin. This experimental fact is correctly predicted by MM2 calculations. Therefore, the combined use of NOE measurements and MM2 calculations offers a possibility to discriminate between these two diastereoisomeric glycosides. Moreover, it is shown, that in the present case meaningful results can also be obtained when the MM2 calculations are carried out on simple model compounds. It is expected that such an approach might also be useful in other cases, where the discrimination of diastereoisomeric open-chain compounds presents a problem.

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References

- [1] D. Neuhaus and M. Williamson, The Nuclear Overhauser Effect in Structural and Conformational Analysis, VCH, New York, 1989.
- [2] D. Neuhaus and M. Williamson, The Nuclear Overhauser Effect in Structural and Conformational Analysis, VCH, New York, 1989, pp 386-420.

Table 8

- [3] J. Tropp, J. Chem. Phys., 72 (1980) 6035-6043.
- [4] S. Maasamune P. Ma, R. Moore, T. Fujiyoshi, C. Jaime, and E. Osawa, J. Chem. Soc., Chem. Commun., (1986) 261-263.
- [5] W. Hübel and A. Nahrstedt, Phytochemistry, 14 (1975) 2723-2725.
- [6] W. Hübel and A. Nahrstedt, Phytochemistry, 17 (1978) 314-315.
- [7] N. Erb, H.D. Zinsmeister, G. Lehmann, and A. Nahrstedt, Phytochemistry, 18 (1979) 1515-1517.
- [8] R. Cook, N. McCaig, J.M.B. McMillan, and W.B. Lumsden, J. Inst. Brew., 96 (1990) 233-244.
- [9] J.W. Jaroszewski, J. Nat. Prod., 49 (1986) 927-928.
- [10] D. Marion and K. Wüthrich, Biochem. Biophys. Res. Commun., 113 (1983) 967-974.
- [11] G. Bodenhausen and D.J. Ruben, Chem. Phys. Lett., 69 (1980) 185.
- [12] A.L. Davis, J. Keeler, E.D. Laue, and D. Moskau, J. Magn. Reson., 98 (1992) 207-216.
- [13] M.F. Summers, L.G. Marzilli, and A. Bax, J. Am. Chem. Soc., 108 (1986) 4285-4294.
- [14] W. Willker, D. Leibfritz, R. Kerssebaum, and W. Bermel, Magn. Reson. Chem., 31 (1993) 287-292.
- [15] D. Neuhaus, J. Magn. Reson., 53 (1983) 109-114.
- [16] M. Kinns and J.K.M. Sanders, J. Magn. Reson., 56 (1984) 518-520.
- [17] N.L. Allinger and Y.H. Yu, Qantum Chemical Program Exchange, 12 (1980) 395.
- [18] J. Castells, C. Jaime, F. López-Calahorra, N. Santaló, and D. Velasco, J. Org. Chem., 53 (1988) 5363-5366.
- [19] P.D.J. Grootenhuis and C.A.G. Haasnoot, Molecular Simulation, 10 (1993) 75-95.