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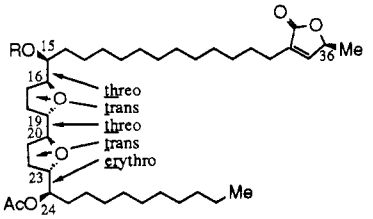
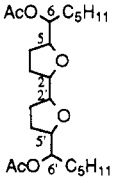
Validation of the ^{1}H NMR Chemical Shift Method for Determination of Stereochemistry in the Bis(tetrahydrofuranyl) Moiety of Uvaricin-Related Acetogenins from Annonaceae: Rolliniastatin 1 (and Asimicin)

Summary: A valuable new method for quantitative correlation of the ^{1}H NMR chemical shift data from the appropriate members (**a**, **g**) of a set of model diastereomeric bis(tetrahydrofurans) (**2a–l**) with those from the triacetate derivative of rolliniastatin 1 (**5**) was developed.

Sir: Unique methodology for determining the relative stereochemical relationship among the six stereogenic carbon atoms in the bis(tetrahydrofuranyl) portion [i.e., C(15)–C(24)] of the antitumor agent uvaricin (**1**)[2] was recently described by us.[3] That process involved, first, the synthesis of 12 diastereomers of the model bis(acetates) **2** having known stereorelationships (because of the methods used in their synthesis)[3,4] and, second, a careful comparison of the chemical shifts observed in the ^{1}H NMR spectra of **2** with those of the acetate derivative of uvaricin (**3**). While we are quite confident in the conclusions we could draw, i.e., the relative stereochemistry indicated in **1**, we recognized that "it is difficult to decide what constitutes a *proof* of stereochemistry by [this class] of arguments".[2] Given that this emerging family of natural products shows potential of having substantial biological significance; given that, with the important exception noted below, it has not been possible to effect X-ray crystallographic analysis of any of these "waxy", "microcrystalline", or "amorphous" natural materials or their derivatives; and given the natural skepticism in our methodology that has been expressed to us by some who are not experienced in chemical shift analysis within series of diastereomeric components, we sought an opportunity to further validate our methodology.



1 R = H, *3* R = Ac } or C(15)-C(24) hexepimer



2

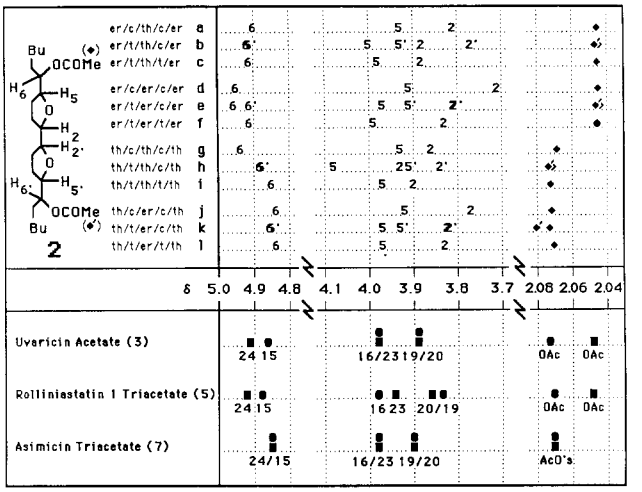
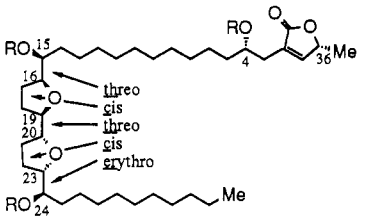
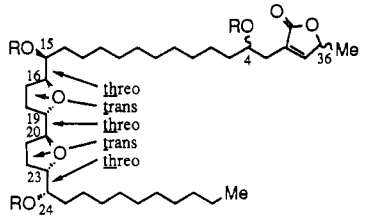


Figure 1. Proton NMR chemical shifts of diagnostic protons in the 12 isomeric dibutylated diacetates (**2**) and of uvaricin acetate (**3**), rolliniastatin triacetate (**5**), and asimicin triacetate (**7**).[11]

Prior to our work the only stereochemical feature reported for any of the 10 or so known compounds of this class was the absolute configuration of the methyl-bearing C(36) in uvaricin and desacetyluvaricin, which was determined as *S* by degradation to lactic acid.[5] Recently, Pettit and co-workers have described their success in determining the first X-ray crystallographic analysis of a derivative of a natural product from this class, rolliniastatin 1 (**4**).[6] The availability of this first family member of known relative configuration prompted us to use it as a test case for our method. Rolliniastatin 1 (**4**)[7] was converted to its triacetate derivative **5** by simple treatment with Ac_2O in pyridine at room temperature.



4 R = H, *5* R = Ac } or enantiomer



6 R = H, *7* R = Ac } or C(15)-C(24) hexepimer

Asimicin (**6**) is yet another member of this family whose constitution (but not stereochemistry) and cytotoxic and pesticidal[8] activities were described by the McLaughlin

(1) Fellow of the Alfred P. Sloan Foundation, 1985–89.
(2) Jolad, S. D.; Hoffmann, J. J.; Schram, K. H.; Cole, J. R.; Tempesta, M. S.; Kriek, G. R.; Bates, R. B. *J. Org. Chem.* **1982**, *47*, 3151.
(3) Hoye, T. R.; Suhadolnik, J. C. *J. Am. Chem. Soc.* **1987**, *109*, 4402.
(4) (a) Hoye, T. R.; Suhadolnik, J. C. *J. Am. Chem. Soc.* **1985**, *107*, 5312. (b) Hoye, T. R.; Suhadolnik, J. C. *Tetrahedron* **1986**, *42*, 2855.
(5) Jolad, S. D.; Hoffmann, J. J.; Cole, J. R.; Barry, C. E. III; Bates, R. B.; Linz, G. S. *J. Nat. Prod.* **1985**, *48*, 644.
(6) The X-ray analysis was performed on the 15-*O*-(*p*-bromophenyl)-urethane derivative of **4**: Pettit, G. R.; Cragg, G. M.; Polonsky, J.; Herald, D. L.; Goswami, A.; Smith, C. R.; Meretti, C.; Schmidt, J. M.; Weisleder, D. *Can. J. Chem.* **1987**, *65*, 1433.
(7) A generous sample of rolliniastatin 1 (**4**) was kindly provided to us by Dr. Cecil R. Smith from Professor Pettit's laboratory. We are indebted for their assistance.

0022-3263/88/1953-5578$01.50/0

laboratory.[9] It is convenient that the ^1H NMR data for the triacetate derivative of asimicin (**7**) were also recorded, and we will comment upon the interpretation of those data[10] in the discussion to follow.

In Figure 1 are accurately graphed the diagnostic proton chemical shifts for the 12 model bis(acetates) **2a–l** [containing the indicated threo (th) or erythro (er) and cis (c) or trans (t) relationships between vicinal stereogenic carbons and across single tetrahydrofuran rings, respectively], uvaricin acetate (**3**), rolliniastatin 1 triacetate (**5**), and asimicin triacetate (**7**).[11] Our previous correlation of uvaricin acetate (**3**) and **2a–l** had relied upon simple visual inspection of the data, which indicated that the best fit occurred between **3** and the two symmetrical model isomers **2c** and **2i**. Thus **3** [and, therefore, uvaricin (**1**)] was assigned the er/t/th/t/th stereochemistry indicated in structures **1** and **3**.

A similar qualitative analysis of the asimicin triacetate (**7**) data shows an excellent match with the chemical shifts of the model isomer **2i**. Moreover, the near identity of the shifts for proton pairs 15/24, 16/23, and 19/20 in **7** strongly suggests that asimicin possesses pseudosymmetric stereochemistry (i.e., identical C(15)/C(16) to C(23)/C(24) and C(16)/C(19) to C(20)/C(23) stereorelationships).[6] Thus, **7** [and, therefore, asimicin (**6**)] can be assigned as having the indicated th/t/th/t/th relative stereochemistry along the bis(THF) backbone.[10]

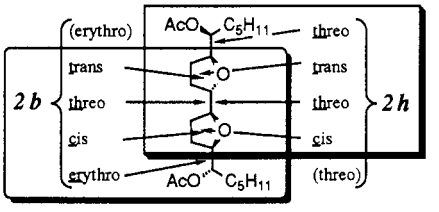
The known stereochemistry of rolliniastatin 1 (**4**)[6] is embodied in the two model diastereomers **2a** and **2g**. Accordingly, if there is merit to our method, the proton chemical shifts of these two would be expected to match those of rolliniastatin 1 triacetate (**5**). The simple inspection analysis, which was used in the uvaricin and asimicin assignments, reveals (see Figure 1) a reasonably good correlation between the data for **5** and **2a** and **2g**. However, there is another pair, **2a** and **2i**, whose chemical shifts also appear to offer a reasonably good match with those of **5**. It was therefore deemed necessary to develop a more sophisticated, quantitative method for analysis of the data.

Twenty possible diastereomers of constitution **2** exist. These are listed by their stereochemical descriptors in Table I. The 12 having identical erythro (er) or threo (th) relationships between C(6)/C(5) and C(6′)/C(5′) are **2a–l** and can be depicted by the descriptors associated with entries 1–12 in Table I. Entries 13–20 represent the eight possible isomers that have one erythro and one threo terminal relationship. None of these has been synthesized, but the anticipated ^1H NMR shifts for each can be extrapolated from judicious combination of the most appropriate pair of isomers from **2a–l**. For example, the unknown th/t/th/c/er isomer (entry 15) can be best represented by the compounds **2h** (th/t/th/c/th) and **2b** (er/t/th/c/er), each of which corresponds to four contiguous of these five stereorelationships.

Table I. Compilation of the Sums of the Differences in Chemical Shifts of Protons 6/5/2/2′/5′/6′ in the Best Models for the 20 Possible Diastereomers of 2 and 15/16/19/20/23/24 in the Peracetates 3, 5, and 7

			$\Sigma\lvert\Delta\delta$'s$\rvert$[c]		
entry	descriptor[a]	model[b]	**3**	**5**	**7**
1	er/c/th/c/er	**2a/2a**	(0.30)	(0.11)	0.36
2	er/t/th/c/er	**2b**	(0.28)	(0.17)	0.52
3	er/t/th/t/er	**2c/2c**	(0.08)	(0.21)	0.16
4	er/c/er/c/er	**2d/2d**	(0.62)	(0.41)	0.72
5	er/t/er/c/er	**2e**	(0.34)	(0.15)	0.44
6	er/t/er/t/er	**2f/2f**	(0.18)	(0.19)	0.28
7	th/c/th/c/th	**2g/2g**	(0.26)	(0.19)	0.36
8	th/t/th/c/th	**2h**	(0.32)	(0.23)	0.32
9	th/t/th/t/th	**2i/2i**	(0.12)	(0.29)	**0.02**
10	th/c/er/c/th	**2j/2j**	(0.44)	(0.27)	0.38
11	th/t/er/c/th	**2k**	(0.28)	(0.15)	0.22
12	th/t/er/t/th	**2l/2l**	(0.22)	(0.21)	0.16
13	er/c/th/c/th	**2a/2g**	0.26	**0.09**	(0.36)
14	er/t/th/c/th	**2b/2h**	0.19	0.15[d]	(0.27)
15	th/t/th/c/er	**2h/2b**	0.35	0.26[d]	(0.41)
16	er/t/th/t/th	**2c/2i**	**0.05**	0.22	(0.09)
17	er/c/er/c/th	**2d/2j**	0.47	0.30	(0.54)
18	er/t/er/c/th	**2e/2k**	0.26	0.19[d]	(0.34)
19	th/t/er/c/er	**2k/2e**	0.26	0.16[d]	(0.32)
20	er/t/er/t/th	**2f/2l**	0.14	0.17	(0.22)

[a] Stereochemical descriptor of each of the 20 possible diastereomers of constitution **2**. [b] Best possible data set for predicting the chemical shifts of the various isomers represented by the descriptors.[a] [c] Sum of the absolute values of the chemical shift differences in ppm between protons 6, 5, 2, 2′, 5′, and 6′ of the models[b] and 15, 16, 19, 20, 23, and 24 of the naturally derived peracetates **3**, **5**, and **7**, respectively. [d] There is ambiguity in how to do the matching of the pair of central protons in this case. The better of the two possible fits was used; thus, this number represents the lower limit for this $\Sigma\lvert\Delta\delta$'s$\rvert$.



Entry 15 = *2h* + *2b*

Having identified the appropriate models for each of the 20 possible isomers of **2** as also noted in Table I, we then determined the sum of the absolute values of the differences in the chemical shifts for protons 15/16/19/20/23/24 in the peracetates **3**, **5**, and **7** and protons 6/5/2/2′/5′/6′ in the models (**2**), respectively. These are recorded as $\Sigma\lvert\Delta\delta$'s$\rvert$ values in Table I. The sums within parentheses are those from potential matches that could with high confidence be ruled out in advance but are included for the sake of completeness. Thus, the proton chemical shifts of the acetate methyl groups in **2a–f** and **2g–l** (all containing terminal either erythro or threo relationships, respectively) all fall within a very narrow range (δ 2.051 ± 0.007 or 2.075 ± 0.006, respectively). The appearance of acetate resonances within each of these ranges for compounds **3**, **5**, and **7** (see Figure 1) allowed an initial determination that the terminal stereorelationships were one erythro and one threo in **3** and **5** and both threo in **7**.[13]

(8) McLaughlin, J. L. U.S. Patent 4 721 727, 1988.
(9) Rupprecht, J. K.; Chang, C.; Cassady, J. M.; McLaughlin, J. L.; Mikolajczak, K. L.; Weisleder, D. *Heterocycles* **1986**, *24*, 1197.
(10) By analysis of the data for **2a–l**[3] vis-a-vis **7**, Professor Jerry L. McLaughlin has previously reached the same conclusion that we report here regarding asimicin stereochemistry (private communication).
(11) Proton chemical shifts recorded in $CDCl_3$ at 300 MHz for **2a–l**, **3**, **5**, and **7** reported as δ (proton no.). **2a**: 4.90 (6), 3.94 (5), and 3.81 (2). **2b**: 4.91 (6), 4.01 (5), 3.88 (2), 3.76 (2′), 3.93 (5′), and 4.91 (6′). **2c**: 4.91 (6), 3.98 (5), and 3.88 (2). **2d**: 4.95 (6), 3.91 (5), and 3.71 (2). **2e**: 4.96 (6), 3.97 (5), 3.81 (2), 3.80 (2′), 3.91 (5′), and 4.91 (6′). **2f**: 4.92 (6), 3.99 (5), and 3.84 (2). **2g**: 4.94 (6), 3.93 (5), and 3.86 (2). **2h**: 4.88 (6), 4.08 (5), 3.93 (2), 3.84 (2′), 3.91 (5′), and 4.88 (6′). **2i**: 4.85 (6), 3.97 (5), and 3.90 (2). **2j**: 4.84 (6), 3.93 (5), and 3.77 (2). **2k**: 4.85 (6), 3.97 (5), 3.82 (2), 3.82 (2′), 3.93 (5′), and 4.85 (6′). **2l**: 4.84 (6), 3.97 (5), and 3.84 (2). **3**: 4.92 (24), 3.98 (23), 3.89 (20), 3.89 (19), 3.98 (16), and 4.86 (15). **5**: 4.91 (24), 3.92 (23),[12] 3.85 (20 or 19), 3.81 (19 or 20), 3.96 (16),[12] and 4.88 (15). **7**:[9] 4.85 (24), 3.98 (23), 3.90 (20), 3.90 (19), 3.89 (16), and 4.85 (15).
(12) Assignment made on the basis of a homonuclear COSY experiment.
(13) These same conclusions can be drawn from analysis of the carbinol methine proton chemical shift data at positions 6/6′ vis-a-vis 15/24 in the alcohol precursors to **2a–l** vs **1**, **4**, and **6**.

It was satisfying to see that, in the uvaricin and asimicin cases (i.e., **3** and **7**), the entries with the lowest $\Sigma|\Delta\delta$'s| values (16 and 9) corresponded to the stereochemistries (i.e., er/t/th/t/th and th/t/th/t/th) that had been assigned by simple inspection (vide supra). Even more rewarding was the fact that, for the case of rolliniastatin 1 triacetate (**5**), the entry with the lowest $\Sigma|\Delta\delta$'s| value (13) corresponded to the stereochemistry (er/c/th/c/th) known[6] for rolliniastatin 1 (**4**). It is clear from the relative magnitudes of the sums of the $|\Delta\delta$'s| that the more quantitative analysis developed and described here was necessary to reach a conclusive decision regarding the "best match" for rolliniastatin 1 triacetate (**5**). The fact that the stereochemistry so identified is identical with that known for rolliniastatin 1 lends considerable validity to this type of approach to structure determination. It is likely that other opportunities to apply this method to new members of this group of bis(tetrahydrofuranyl) acetogenins will present themselves. We predict that, more often than not, this method will be the only way to access this stereochemical knowledge.

Acknowledgment. This investigation was supported by Grant GM-34492 awarded by the DHHS and by an award from the Alfred P. Sloan Foundation.

Registry No. **2a**, 109215-01-2; **2b**, 109215-02-3; **2c**, 109215-03-4; **2d**, 109215-04-5; **2e**, 109215-05-6; **2f**, 109215-06-7; **2g**, 109215-07-8; **2h**, 109215-08-9; **2i**, 109215-09-0; **2j**, 109215-10-3; **2k**, 109215-11-4; **2l**, 109215-12-5; **3**, 109122-75-0; **4**, 111056-97-4; **5**, 116865-22-6; **6**, 102989-24-2; **7**, 116947-15-0.

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Preferred Conformation of *C*-Glycosides. 5. Experimental Support for the Conformational Similarity between *C*- and *O*-Disaccharides

Summary: Experiments demonstrating the conformational similarity between the *C*-disaccharides **2**, **5**, and **8** and the corresponding parent *O*-disaccharides **1**, **4**, and **7** are reported.

Sir: We have recently developed and provided experimental support for a model to predict the preferred conformations of *C*-glycosides, based on the analysis of steric interactions primarily around the nonglycosidic bond.[1] In the interest of extending its predictive value to the corresponding parent glycosides, we have undertaken studies to compare the conformational preferences of representative *C*- and *O*-disaccharides. In this paper we present the results of our investigations.

The methyl *C*-disaccharides **2**, **5**, and **8**[1e] and the corresponding oxygen-linked systems **1**, **4**, and **7**[2] were chosen for study. On the basis of our model, each of these compounds is expected to exist predominantly in one conformation in the ground state. In order to compare the two series, recourse was made to nuclear Overhauser effects (NOE) and T_1 measurements. These techniques have been used extensively in the conformational analysis of oligosaccharides.[3] However, we must emphasize that we are not attempting to use these methods as the principal means of conformational analysis. Experimentally, we *first* establish the conformational preference of the carbon series, relying on the values of vicinal spin–spin coupling constants, and *second* compare the conformational behavior of *O*- and *C*-disaccharides through NOE and T_1 data.

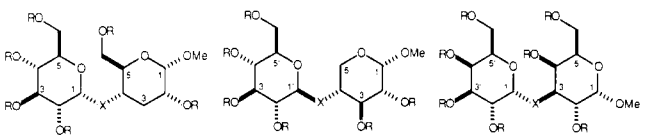
Table I. Nuclear Overhauser Effect (NOE) Data at Room Temperature

	enhancement (%)[a]		
	1′,3(eq)[b]	1′,4	3(eq),1′
1a	6.9	7.2	8.7
2a	5.1	0.0	6.1
3a	5.2	3.1	9.1
1b	4.6	5.3[c]	8.0
2b	3.2	0.0	5.1
3b	5.1	5.1	6.3

	enhancement (%)[a]					
	1′,5(eq)	1′,4	5(eq),1′	4,1′	4,2′	2′,4
4a	4.8	12.1[d]	11.7[e]	*f*	*f*	3.4[d]
5a	5.1	4.3	4.8	1.9	1.6	*f*
6a	5.7	5.9	5.9	3.4	3.8	4.8
4b	NOE[g]	5.2	*f*	5.3	*f*	*f*
5b	*f*	*f*	4.6[h]	4.2[h]	*f*	*f*
6b	*f*	*f*	2.9[h]	2.3[h]	*f*	*f*

	enhancement (%)[a]		
	1′,3	1′,4	4,1′
7a	4.0	3.5	4.8
8a	1.7	3.2	5.5
7b	5.7	2.9	3.1
8b	1.2	2.5	2.7

[a] NOE experiments were performed in CD_3OD for **1a**, **2a**, **3a**, **4a**, **5a**, and **6a**, in $CDCl_3$ for **1b**, **2b**, **3b**, and **4b**, in C_6D_6 for **5b**, **6b**, **7b**, and **8b**, and in D_2O containing 13% pyridine-d_5 for **7a** and **8a**. [b] This notation indicates that an NOE enhancement of H-3(eq) was observed on irradiation of H-1′. [c] H-4, H-5(5′), and H-3′ overlap. NOE enhancement of H-5(5′) and H-3′ could not be excluded. [d] H-3, H-4, and H-6′ overlap. NOE enhancement of H-3 and H-6′ could not be excluded. [e] Partial irradiation of H-4 may contribute to the magnitude of the observed NOE. [f] This data is not available. [g] H-5(eq), H-3′, H-4′, H-6a′, and H-6b′ overlap. A 5.4% enhancement within this multiplet was observed and was assigned to H-3′ and H-5(eq). NOE enhancement of H-4′, H-6a′, and H-6b′ could not be excluded. [h] H-1′, H-2′, H-5′, and H-OMe overlap. NOE enhancement of H-2′, H-5′, and H-OMe could not be excluded.



1a: X=O, R=H
1b: X=O, R=Bn

2a: X=CH_2, R=H
2b: X=CH_2, R=Bn

3a: X=CD_2, R=H
3b: X=CD_2, R=Bn

4a: X=O, R=H
4b: X=O, R=Bn

5a: X=CH_2, R=H
5b: X=CH_2, R=Bn

6a: X=CD_2, R=H
6b: X=CD_2, R=Bn

7a: X=O, R=H
7b: X=O, R=Bn

8a: X=CH_2, R=H
8b: X=CH_2, R=Bn

(1) (a) Babirad, S. A.; Wang, Y.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 1370. (b) Wu, T.-C.; Goekjian, P. G.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4819. (c) Goekjian, P. G.; Wu, T.-C.; Kang, H.-Y.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4823. (d) Babirad, S. A.; Wang, Y.; Goekjian, P. G.; Kishi, Y. *J. Org. Chem.* **1987**, *52*, 4825. (e) Wang, Y.; Goekjian, P. G.; Ryckman, D. M.; Kishi, Y. *J. Org. Chem.* **1988**, *53*, 4151.

(2) These were prepared by known literature methods; see: Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. Schemes for the syntheses of **1a,b**, **4a,b**, and **7a,b** are included in the supplementary material. All the new compounds reported in this paper gave satisfactory spectroscopic data, including ^1H NMR, IR, and MS.

(3) NOE and T_1 data have been used extensively in the conformational analysis of saccharides. For recent reviews, see: (a) Williams, N, R.; Davison, B. E.; Ferrier, R. J.; Furneaux, R. H. *Carbohydr. Chem.* **1985**, *17*, 205. (b) Carver, J. P.; Brisson, J.-R. In *Biology of Carbohydrates*, Vol. II; Ginsburg, V., Robbins, P. W., Eds.; John Wiley and Sons: New York, 1984; p 289. (c) Bock K. *Pure Appl. Chem.* **1983**, *55*, 605. (d) Bock, K.; Thogersen, H. *Annu. Rep. NMR Spectrosc.* **1982**, *13*, 1.

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