ORGANIC LETTERS

2004
Vol. 6, No. 18
3019−3022

Chiral Silylation Reagents: Determining Configuration via NMR-Spectroscopic Coanalysis

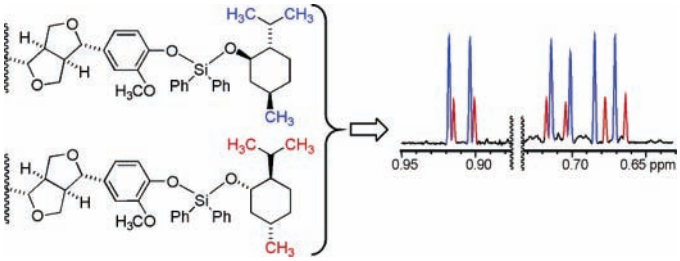
Frank C. Schroeder, Douglas B. Weibel, and Jerrold Meinwald*

Department of Chemistry and Chemical Biology, Cornell University, Ithaca, New York 14853-1301

circe@cornell.edu

Received April 27, 2004 *(Revised Manuscript Received June 30, 2004)*

ABSTRACT



Derivatization with (+)- and (–)-chloromenthoxydiphenylsilane was used to determine the absolute configuration of the insect defensive agent pinoresinol (1). Although the ^{1}H NMR chemical shift differences of the resulting two diastereomers are small, ^{1}H NMR spectroscopy provided for the unambiguous assignment of the natural product's configuration. For this purpose, a new approach involving NMR spectra of mixtures of diastereomers was used. Our method resembles coinjecting mixtures of samples of known and unknown configuration in GC and HPLC.

One of the most widely used techniques for determining the absolute stereochemistry of natural products involves the incorporation of a chiral derivatizing agent (CDA) and subsequent analysis via NMR spectroscopy.[1] These methods frequently involve the introduction of a chiral substituent directly at or in close proximity to the chiral center of interest to achieve complete separation of corresponding NMR signals in the two complementary diastereomers. While complete separation is desirable, it is often not possible to introduce a chiral substituent sufficiently close to the chiral center to achieve such a high degree of chemical shift separation, especially if the chiral center is not at or next to a hydroxy or amino group. Using the comparatively much smaller chemical shift differences induced by derivatizing a chiral, conformationally flexible molecule at a location far away from the chiral centers of interest seems less obvious.[2] In this situation, corresponding signals in the ^{1}H NMR spectra of the resulting complementary diastereomers would usually not be well separated, although the respective chemical shift values might be somewhat different.[3] We here report the determination of the absolute configuration of a natural product, pinoresinol (**1**), via derivatization with a chiral silylating agent at the periphery of the molecule, using the resulting minute ^{1}H NMR chemical shift differences for unambiguous stereochemical assignment.

We recently identified pinoresinol (**1**) as a minor component in the defensive secretion obtained from larvae of the European cabbage butterfly, *Pieris rapae*.[4] This finding was somewhat surprising because pinoresinol, though widely

(1) (a) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512−519. (b) Seco, J. M.; Quiñoá, E.; Riguera, R, *J. Org. Chem.* **1999**, *64*, 4669−4675. (c) Kato, N. *J. Am. Chem. Soc.* **1990**, *112*, 254−257. (d) Anderson, R. C.; Shapiro, M. J. *J. Org. Chem.* **1984**, *49*, 1304−1305. (e) Doolittle, R. E.; Heath, R. R. *J. Org. Chem.* **1984**, *49*, 5041−5050. (f) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 1092−4096. For a recent review, see: Seco, J. M.; Quinoa, E.; Riguera, R. *Chem. Rev.* **2004**, *104*, 17−117.

(2) Seco, J. M.; Latypov, Sh.; Quiñoá, E.; Riguera, R. *Tetrahedron* **1997**, *53*, 8541−8564.

(3) For an example for the use of small chemical shift differences to establish remote stereochemical relationships, see: Boyle, C. D.; Kishi, Y. *Tetrahedron Lett.* **1995**, *36*, 5695−5698.

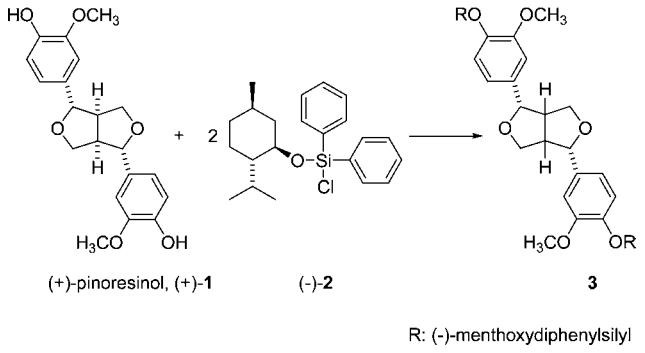
(4) Schroeder, F. C.; Grant, J. B.; Weibel, D. B.; Smedley, S. R.; Bolton, K. L.; Meinwald, J.; Eisner, T. *Proc. Natl. Acad. Sci. U.S.A.*, submitted.

10.1021/ol049233b CCC: $27.50
Published on Web 08/13/2004

occurring in plants,[5] had never been reported as a defensive agent in insects. Accordingly, we found ourselves confronted with the need to determine the absolute configuration of the material isolated from the caterpillar's secretion. The amount of pinoresinol that can be isolated from the secretion is quite small (~100 μg isolated from >2000 larvae) and does not suffice for determining its absolute configuration via chiroptical methods. Furthermore, with only a small amount of material available, we were hesitant to use CDA-based methods that would require multiple steps, such as a partial degradation of pinoresinol (**1**) to expose hydroxy groups closer to the stereocenters in **1** and their subsequent derivatization. Instead, we considered directly derivatizing the phenolic hydroxy groups in **1** with a CDA, hoping this would allow us to differentiate the resulting diastereomers by NMR.

Given the large intramolecular distance between the phenolic hydroxy groups and the chiral centers in pinoresinol, only very small differences in the chemical shift values for any pair of diastereomers arising from such a derivatization can be expected. It seemed highly unlikely that one could achieve complete separation of corresponding NMR signals in the spectra of complementary diastereomers with either commonly available CDAs or our recently described chiral silylation reagents.[6] Nevertheless, since silylation can be achieved under mild conditions and works well even for trace amounts of material, we decided to derivatize a sample of authentic (+)-pinoresinol, isolated from *Forsythia suspensa* leaves,[7] with the (+)- and (−)-isomers of chloromenthoxydiphenylsilane, **2** (Scheme 1). The phenolic hydroxy groups reacted smoothly, and the desired diastereomeric derivatives **3** and **4** were produced in good yields.

Scheme 1. Derivatization of (+)-Pinoresinol



The ^{1}H NMR spectra show minute differences in the chemical shift values of the two diastereomers **3** and **4**. Most prominently, the doublets corresponding to the methyl groups in the menthoxy substituents appear at slightly different chemical shift values (Figure 1). In terms of the size of the chemical shift difference observed, using benzene-d_6 as the solvent gave the best results, as had been the case in earlier applications of the silylation technique.[6a] Next, a sample of pinoresinol (100 μg) isolated from cabbage butterfly caterpillars was derivatized with (−)-menthoxydiphenylsilyl chloride. As shown in Figure 1, the ^{1}H NMR spectrum of the caterpillar derived silyl ether (**5**) resembled that of the (+)-menthoxydiphenylsilyl derivative of (+)-pinoresinol (**4**) more closely than that of its diastereomer **3**, thus suggesting that the caterpillar secretes (−)-pinoresinol. However, the chemical shift differences observed are extremely small, generally of the order of 0.004 ppm and smaller (2 Hz or less at 500 MHz). Chemical shifts are often not reproducible at this level, because many factors such as sample concentration, water content, or pH can influence the exact values in a difficult to predict manner. Therefore, a mere comparison of spectra does not allow for unambiguous assignment of configuration in cases such as the pinoresinol derivatives **3** and **4** where spectra show only minute differences.

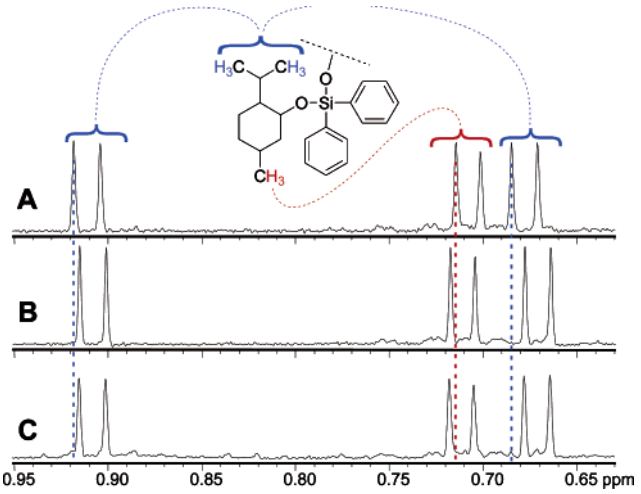


Figure 1. 0.65−0.95 ppm section of ^{1}H NMR spectra (benzene-d_6, 500 MHz): (A) (−)-menthoxydiphenylsilyl derivative of (+)-pinoresinol (**3**); (B) (+)-menthoxydiphenylsilyl derivative of (+)-pinoresinol (**4**); (C) (−)-menthoxydiphenyl-silyl derivative of pinoresinol isolated from cabbage butterfly caterpillars (**5**).

To neutralize the variability of chemical shift resulting from the above-mentioned factors, we introduced a simple modification to our protocol for NMR-based stereochemical assignment. Instead of relying on chemical shift values relative to a reference such as the TMS or the solvent signal, only chemical shift differences between the two diastereomers observed in samples of mixtures of the two were considered. This approach removes the effect of chemical shift variability caused by sample-specific factors since not only are both diastereomers affected in the same way, but more importantly, mixing two diastereomers there are only two possible outcomes: either there are two sets of signals

(5) Kasahara, H.; Miyazawa, M.; Kameoka, H. *Phytochemistry* **1997**, *44*, 1479−1482.

(6) (a) Weibel, D. B.; Walker, T. R.; Schroeder, F. C.; Meinwald, *J. Org. Lett.* **2000**, *2*, 2381−2383. (b) Williamson, R. T.; Barrios Sosa, A. C.; Mitra, A.; Seaton, P. J.; Weibel, D. B.; Schroeder, F. C.; Meinwald, J.; Koehn, F. E. *Org. Lett.* **2003**, *5*, 1745−1748.

(7) Kitagawa, S.; Hisada, S.; Nishibe, S. *Phytochemistry* **1984**, *23*, 1635−1636.

in the ^{1}H NMR spectra or just one. In fact, the absolute magnitude of the chemical shift differences is of little importance when following this approach, as long as there are differences the spectrometer is able to resolve.

In preparation for the mixing experiments, NMR samples of **3** and **4** were prepared with concentrations matching that of the caterpillar-derived sample. Subsequently, spectra of mixtures containing **3** and **4** in a ratio of 1:2 and 2:1 were acquired. These spectra were then used as reference spectra for the subsequent analysis of the caterpillar-derived material (**5**), which was divided into two portions. Of these, one was added to the sample of pure **3** and the other one was added to the sample of pure **4**. The results are shown in Figure 2.

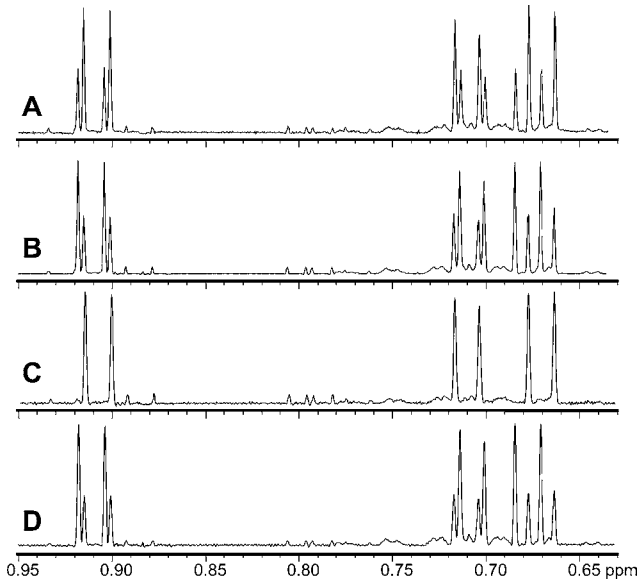


Figure 2. 0.65−0.95 ppm section of ^{1}H NMR spectra (benzene-d_6, 500 MHz): (A) and (B) mixtures of **3** and **4** in approximate ratios of 1:2 and 2:1, respectively; (C) mixture of (+)-menthoxydiphenylsilyl derivative of (+)-pinoresinol (**4**) and **5**; (D) mixture of (−)-menthoxydiphenylsilyl derivative of (+)-pinoresinol (**3**) and **5**.

While the mixture of caterpillar derived material (**5**) and **4** shows just one set of ^{1}H NMR signals (Figure 2C), there are clearly two sets of signals in the spectrum of the mixture containing **3** (Figure 2D). Since **5** was obtained through derivatization with (−)-menthoxydiphenylsilyl chloride, it must be the enantiomer of **4**, thus unambiguously defining the material isolated from the caterpillar secretion as (−)-pinoresinol.

In light of these results, we were interested in whether our method is applicable to other natural products where the site of derivatization is far removed from the chiral center(s). For this purpose, we prepared the (+)- and (−)-isomers of chloromenthoxydiphenylsilane derivatives of *N*-acetyl-L-tyrosine ethyl ester (**6**), (*S*)-citronellol (**7**), (5*S*)-5,9-dimethyl-8-decen-1-ol (**8**), which we prepared from (*S*)-citronellyl bromide,[8] and 11-methyltridecanol (**9**).

In all cases, reactions proceeded smoothly and produced pairs of silylated derivatives in near 100% yield. For (*S*)-citronellol, the chemical shift differences between its two diastereomeric silylation products are quite large (Figure 3A).

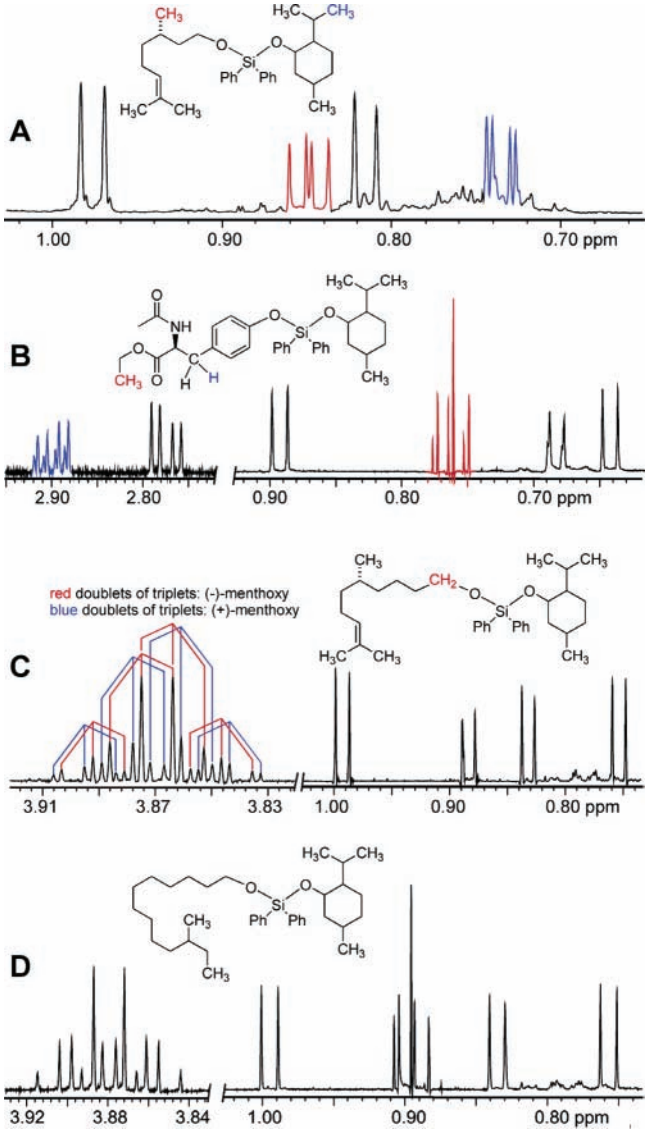


Figure 3. Sections of ^{1}H NMR spectra (benzene-d_6, 500 MHz (A and B) and 600 MHz (C and D)): (A) 1:1 mixture of the (+)- and (−)-**2** derivatives of (*S*)-citronellol (**7**); (B) 2:1 mixture of the (+)- and (−)-**2** derivatives of *N*-acetyl-L-tyrosine ethyl ester (**6**); (C) 1:2 mixture of the (+)- and (−)-**2** derivatives of (5*S*)-5,9-dimethyl-8-decen-1-ol (**8**); (D) 1:1 mixture of the (+)- and (−)-**2** derivatives of 11-methyltridecanol (**9**).

In this case, a straightforward comparison of ^{1}H NMR spectra would suffice in order to assign configuration. For the silyl derivatives of *N*-acetyl-L-tyrosine methyl ester (**6**) and (5*S*)-5,9-dimethyl-8-decen-1-ol (**8**), the chemical shift differences

(8) *S*)-5,9-Dimethyl-9-decen-1-ol (**8**) was synthesized via a short sequence starting with reacting diethyl malonate with (*S*)-citronellyl bromide. For reaction conditions, see: Kletzke, P. G.; *J. Org. Chem.* **1964**, *29*, 1363−1366.

are much smaller, as can be expected since the chiral centers in these derivatives are further removed from each other (Figure 3B,C). Also, it should be noted that in the case of (**8**) the resulting derivatives are conformationally highly flexible. In fact, differences in the ^{1}H NMR spectra of different samples of the (+)-**2** derivative of **8** are of the same order of magnitude as differences between the spectra between the (+)- and (−)-derivatives. Therefore, a mere comparison of the respective ^{1}H NMR spectra would not suffice to unambiguously determine absolute configuration of **8** and might also not be satisfactory for the tyrosine derivative **6**. However, spectra of mixtures of the two diastereomers of **6** and **8** immediately show that, in fact, there are resolvable chemical shift differences (Figure 3B,C), which could be used for configurational assignment as described above for pinoresinol. Considering the conformational flexibility of **8**, determination of its absolute configuration using other NMR-based methods or chromatographic methods such as chiral GC or HPLC would seem very difficult. However, the ultimate limitation for NMR-based differentiation of diastereomers is determined by the resolution of available spectrometers. For example, for a 1:1 mixture of the two diasteromeric silylation products of 11-methyltridecanol (**9**) even a well-resolved ^{1}H NMR spectrum acquired at 600 MHz shows only one set of signals (Figure 3D).[9]

Our simple version of NMR-based stereochemical analysis resembles the common GC or HPLC method of co-injecting an unknown as a mixture with a reference sample. For example, to determine the absolute configuration of a volatile compound by chiral GC,[10] one would usually begin with finding suitable conditions for separating the enantiomers into two peaks. Co-injection of the sample of unknown configuration with samples of either enantiomer would then generate chromatograms showing either two peaks or one peak, which then allows for assignment of configuration in very much the same manner as in the NMR-based procedure described here. Of course, using NMR one does not depend on the separation of just one peak. In most cases, several signals in the NMR spectra of a pair of diastereomers will show chemical shift differences that could be taken advantage of.

In summary, we have shown that extremely small differences in the NMR spectra of pairs of diastereomers can be used for unambiguous assignment of configuration. We imagine that this method will be of great advantage especially in situations where the stereocenter of interest is far removed from the site of derivatization, and thus the differences in ^{1}H chemical shift values are likely to be minute. In the present case, the menthoxy methyl groups responsible for the NMR signals used in this analysis are actually each eleven bonds away from the nearest chiral center in the pinoresinol substrate. In addition, the assignment of the absolute configuration of pinoresinol isolated from *Pieris rapae* represents the first application of our recently introduced chiral silylation reagents to the derivatization of phenols. This method should be particularly useful for the stereochemical analysis of flavonoids and lignans, many of which have important biological activity or are of medicinal interest.[11]

Acknowledgment. This work was supported in part by the National Institutes of Health (GM 53830 and training grant GM 08500). We thank Dr. Andrew E. Taggi for helpful comments. The hospitality of the American Academy of Arts & Sciences to J.M. during the preparation of this manuscript is acknowledged with pleasure.

Supporting Information Available: Procedures for the preparation of **2** and the derivatization of pinoresinol; full spectra of mixtures of derivatives **3**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL049233B

(9) In some cases, the use of ^{13}C NMR spectroscopy might help to further extend the scope of NMR-based assignment of absolute configuration. However, the amounts of material needed would be much higher than when using ^{1}H NMR.

(10) Schurig, V. *TrAC, Trends Anal. Chem.* **2002**, *21*, 647−661.

(11) Lewis, N. G., Davin, L. B. In *Comprehensive Natural Product Chemistry*; Barton, D. H. R., Nakanishi, K., Meth-Cohn, O., Eds.; Elsevier: London, 1999; pp 639−712.