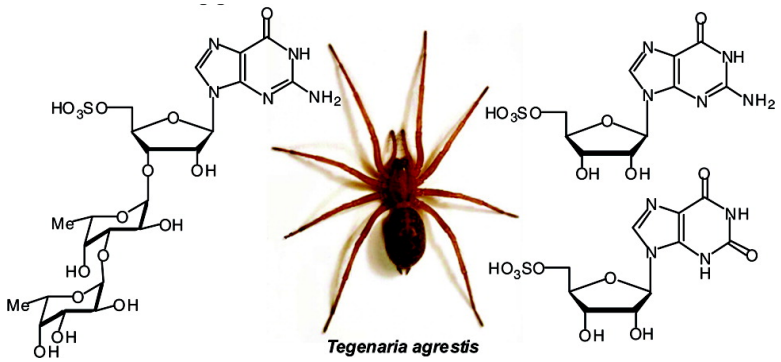
Article

A New Approach to Natural Products Discovery Exemplified by the Identification of Sulfated Nucleosides in Spider Venom

Andrew E. Taggi, Jerrold Meinwald, and Frank C. Schroeder

J. Am. Chem. Soc., **2004**, 126 (33), 10364-10369• DOI: 10.1021/ja047416n • Publication Date (Web): 28 July 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 1 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
ARTICLES
Published on Web 07/28/2004

A New Approach to Natural Products Discovery Exemplified by the Identification of Sulfated Nucleosides in Spider Venom

Andrew E. Taggi, Jerrold Meinwald, and Frank C. Schroeder*

Contribution from The Cornell Institute for Research in Chemical Ecology and The Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University, Ithaca, New York 14853

Received May 3, 2004; E-mail: fs31@cornell.edu

Abstract: Using a new approach based on the NMR spectroscopic analysis of the entire, unpurified spider venom, we identified a family of unusual sulfated nucleoside derivatives from the venom of the hobo spider, *Tegenaria agrestis*. These compounds are ribonucleoside mono- and disulfates derived from guanosine and xanthosine, some of which are glycosylated, bearing one or two D-fucose units. The use of NMR spectroscopy to characterize the unfractionated venom was central to the discovery of this heretofore overlooked class of venom components.

Introduction

With almost 40000 described species, spiders are second only to insects as the most diverse group of animals on land. In attaining this diversity, spiders have evolved sophisticated chemical weapons,[1] which makes them an attractive target for chemical prospecting. Recent drug candidates developed from spider venom components block the neuronal nicotinic acetylcholine receptor,[2] increase parathyroid hormone (PTH) secretion,[3] and inhibit atrial fibrillation, a common chronic cardiac arrhythmia.[4] Spider venoms, like those of other venomous animals, consist of complex mixtures of biologically active compounds. The primary small-molecule toxins are often acylpolyamines (with over 100 structures having been described),[5] though the venom may also contain nucleosides, polypeptides, and proteins (including enzymes), as well as citric acid, monoamines, and free amino acids.[6] Considering the large amount of analytical work on spider venom already published, the recent identification of a member of an entirely new class of spider neurotoxin seemed surprising. Activity-guided screening of the venom of the grass spider, *Hololena curta*, led to the discovery of the unique venom component HF-7 (**1**) (Figure 1), which is a bissulfated glyconucleoside.[7] HF-7 has the uncommon ability to effectively block kainate receptors, in addition to weakly blocking L-type calcium channels.

The discovery of this entirely unexpected natural product suggested to us that spider venoms might still harbor interesting new classes of neurotoxins. Moreover, considering the multitude of acylpolyamines that can be identified from a single species, it seemed unlikely that HF-7 was the only spider venom component of its kind and the question remained as to why sulfated nucleosides had not been found in any other previous analysis. Thus, we initiated a program for the chemical characterization of a diverse sampling of spider venoms using a new NMR-based approach. Central to our analytical approach is the acquisition of a set of NMR spectra of the *entire crude venom* without any prior purification, including at a minimum ^{1}H and (^{1}H,^{1}H)-dqf-COSY spectra.

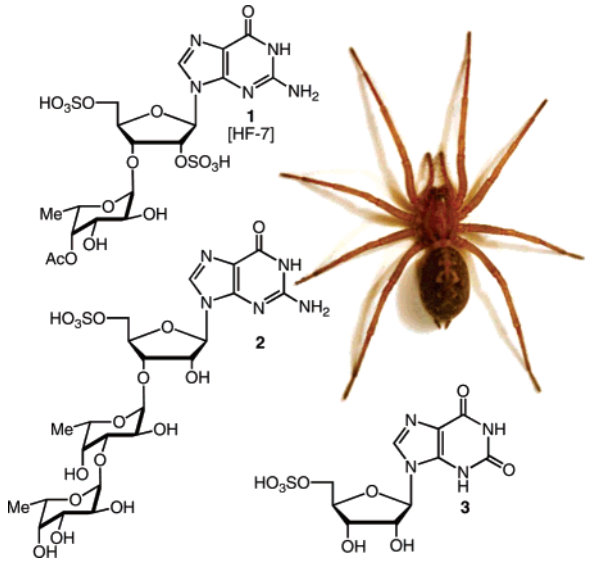
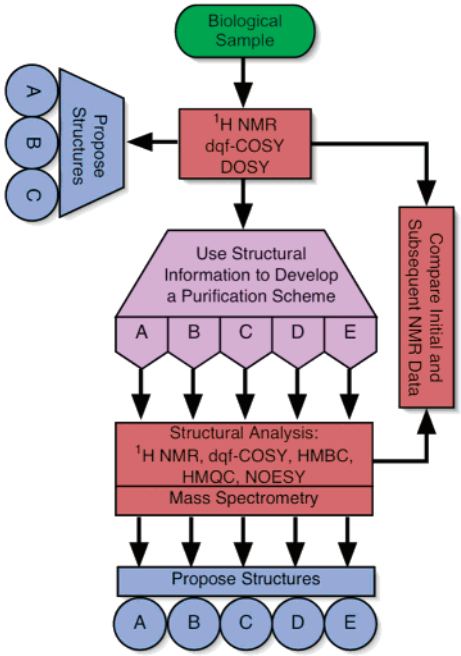


Figure 1. HF-7 (**1**), isolated from *H. curta*, and a photograph of a female *T. agrestis* with examples of sulfated nucleosides (**2**, **3**) identified from its venom.

(1) Platnick, N. I. *Advances in Spider Taxonomy*, 1988−1991; The New York Entomological Society: New York, 1993.
(2) Villarroya, M.; Gandía, L.; López, M. G.; García, A. G.; Cueto, S.; García-Navio, J.-L.; Alvarez-Builla, J. *Bioorg. Med. Chem. Lett.* **1996**, *4*, 1177−1183.
(3) (a) Shu, Y.-Z. *J. Nat. Prod.* **1998**, *61*, 1053−1071. (b) Nemeth, E. F.; Steffey, M. E.; Hammerland, L. G.; Hung, B. C. P.; Van Wagenen, B. C.; DelMar, E. G.; Balandrin, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4040−4045.
(4) Bode, F.; Sachs, F.; Franz, M. R. *Nature* **2001**, *409*, 35−36.
(5) McCormick, K. D.; Meinwald, J. *J. Chem. Ecol.* **1993**, *19*, 2411−2451.
(6) Jackson, H.; Parks, T. N. *Annu. Rev. Neurosci.* **1989**, *12*, 405−414.
(7) McCormick, J.; Li, Y.; McCormick, K.; Duynstee, H. I.; van Engen, A. K.; van der Marel, G. A.; Ganem, B.; van Boom, J. H.; Meinwald, J. *J. Am. Chem. Soc.* **1999**, *121*, 5661−5665.

10.1021/ja047416n CCC: $27.50

Scheme 1. Use of "Direct NMR" for the Identification of Natural Products



We show that the use of NMR spectroscopy to characterize the unpurified venom allows for an *impartial* view of its composition, without any skewing of the results stemming from prepurification (*vide infra*). We demonstrate the efficacy of this approach in the case of the hobo spider, *Tegenaria agrestis*, which led to our identification of a family of no fewer than seven novel nucleoside-derived natural products.

Analytical Methodology

Despite great advances in chromatographic separation technology and analytical instrumentation over the past few decades, the general approach to identification of new natural products has changed very little. This process generally begins with the collection of a large number of specimens, which are then homogenized, lyophilized, and extracted with organic solvents. Subsequent fractionation and characterization of this natural product "soup" is usually motivated by the search for a specific biological activity or sometimes by the suspected presence of novel molecular structures. In this paper, we propose an improved approach to natural products discovery based on direct NMR spectroscopic characterization of the biological material *prior* to any fractionation (Scheme 1).

Apart from the desire to obtain pure compounds for biological testing, isolation of individual compounds is primarily motivated by a perceived need to simplify a mixture prior to structural analysis. Unfortunately, analytical approaches that involve an initial chromatographic step, such as GC or HPLC, are likely to discriminate against some classes of compounds, while favoring others. From our experience, structurally unique compounds will often not survive arbitrarily chosen chromatographic conditions, which is one reason they have remained undescribed.[8] To overcome these difficulties, we suggest employing direct NMR spectroscopic analyses of crude extracts, which provides a much more *impartial* view of the sample's contents, and in many cases will already allow for the partial identification of some of the novel compounds present. We suggest, at the minimum, the acquisition of ^{1}H and (^{1}H,^{1}H)-dqf-COSY spectra. In some cases diffusion-ordered spectroscopy (DOSY) may also prove useful.[9]

Screening for new natural products using direct NMR spectroscopic analyses of crude or partially purified materials has important advantages over solely mass-spectroscopy-based approaches.[10] One major disadvantage of using MS as the primary analytical tool is that the appropriate ionization technique(s) can only be determined once initial structural data are available. Even if the ionization techniques chosen allow for detection of most of the compounds in a complex natural products mixture, the *connectivity* information available through 2D NMR represents an invaluable addition to mass spectroscopic results.[11] Furthermore, any assessment of the quantitative composition of unknown compounds through MS will necessarily be very uncertain. Thus, when choosing an exclusively MS-based approach, one may inadvertently exclude entire new structural classes.

From the initially acquired 1D and 2D NMR spectra of a mixture, sufficient data may be obtained to identify some or all of the components of interest. When this is not the case, the preliminary structural information is used to develop a purification scheme, in such a way as to prevent the unknowns from changing, thus precluding a skewing of the results by the analytical techniques employed. After HPLC separation, the collected fractions are reanalyzed using ^{1}H and dqf-COSY spectra. This information is then compared with the original spectroscopic data to determine if any of the components have undergone degradation or rearrangement. This comparison is essential to determine whether one is identifying natural products rather than degradation products.

One frequent concern when working with biological material suspected to have potent activity is its scarcity. For example, for most spider species the amounts of venom that can be collected are extremely small. Often only a fraction of a microliter of venom can be obtained from one individual. Especially in situations such as this, it seems prudent to acquire all available NMR spectroscopic data prior to any mass spectroscopic analysis, since NMR analysis, as opposed to MS, is nondestructive. With NMR data in hand, the optimal mass spectrometric ionization technique is usually quite apparent, and thus, structural assignments can be easily completed.

Results

Venom of *T. agrestis* was obtained through electrostimulation of the venom gland,[12] which causes the spider to release venom into a capillary placed over its fang. This allows for the collection of a pure sample of venom free from digestive proteases that could potentially degrade some of the venom's components. Our analysis of *T. agrestis* began by dissolving the *entire* lyophilized venom sample (31 mg dry mass corresponding to 235 μL of venom) in D_2O, followed by the acquisition of a ^{1}H NMR spectrum. At first glance, the resulting spectrum looks extremely complicated, as a consequence of multiply overlapping signals covering almost the entire sweep width (Figure 2A). Clearly, this initial ^{1}H NMR spectrum is

(8) For preliminary examples of the use of direct NMR analysis to identify unusual natural products from unfractionated mixtures, see: (a) Schröder, F. C.; Farmer, J. J.; Attygalle, A. B.; Smedley, S. R.; Eisner, T.; Meinwald, J. *Science* **1998**, *281*, 428−431. (b) Schröder, F. C.; Tolasch, T. *Tetrahedron* **1998**, *54*, 12243−12248. (c) Schröder, F.; Sinnwell, V.; Baumann, H.; Kaib, M.; Francke, W. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 77−80. (d) Schröder, F.; Baumann, H.; Kaib, M.; Sinnwell, V. *Chem. Commun.* **1996**, 2139−2140.

(9) (a) Johnson, C. S., Jr. *Prog. Nucl. Magn. Reson. Spectrosc.* **1999**, *34*, 203−256. (b) Morris, K. F.; Johnson, C. S., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 3139−3141.

(10) For examples of the use of NMR spectroscopy of crude materials for the detection and quantification of known compounds, see: (a) Fan, W.-M.; Colmer, T. D.; Lane, A. N.; Higashi, R. M. *Anal. Biochem.* **1993**, *214*, 260−271. (b) Gerhard, U.; Thomas, S.; Mortishire-Smith, R. *J. Pharm. Biomed. Anal.* **2003**, *32*, 531−538.

(11) For example, the identification of the spin systems provides unambiguous evidence for the proposed connectivity of the molecule, while observed NOEs and coupling constants can provide stereochemical information.

(12) (a) Grant, J. B.; Land, B. *Herpetol. Rev.* **2002**, *33*, 38−41. (b) Norment, B. R.; Smith, O. E. *Toxicon* **1968**, *7*, 141−144.

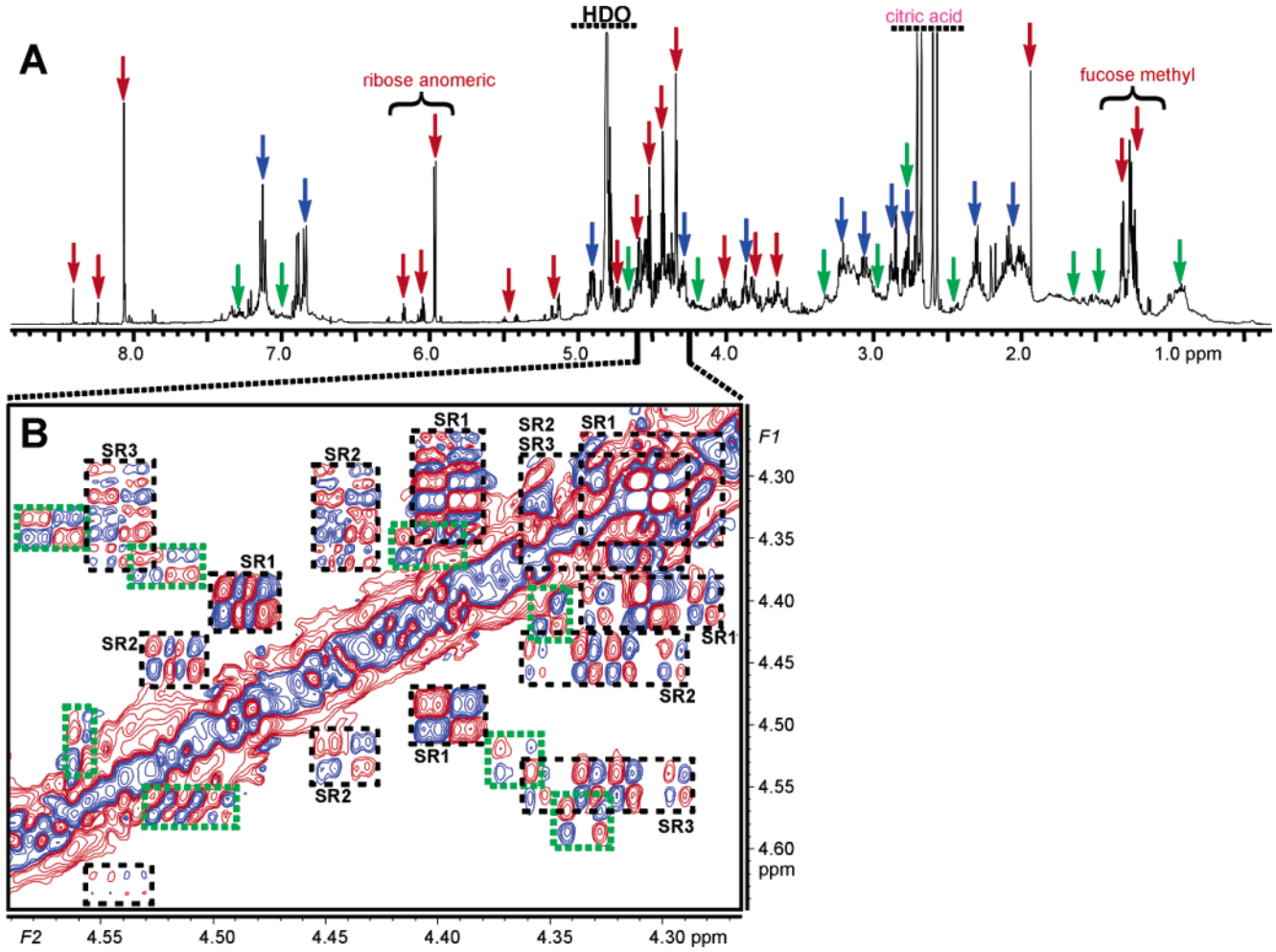


Figure 2. (A) ^{1}H NMR spectrum of lyophilized, unfractionated *T. agrestis* venom in D_2O at 500 MHz. Signals marked in red belong to nucleoside derivatives, signals marked in green correspond to proteins, and signals marked in blue correspond to polyamines. (B) Section of the corresponding (^{1}H,^{1}H) dqf-COSY spectrum. SR1−SR3: cross-peaks of three sulfated ribonucleosides. Cross-peaks labeled SR1 belong to the major nucleoside in the secretion, guanosine-5′-sulfate (**5**). Cross-peaks marked in green belong to polyamines and peptides. At lower threshold, cross-peaks of additional spin systems become visible, among those, several corresponding to additional sulfated riboses.

not suited to compound identification. Its main value consists, rather, in providing a record or *fingerprint* of the original composition of the natural material. In addition, it might contain hints for the presence of unusual small molecules.

NMR signals derived from small molecules generally tend to be well resolved, standing out from those of proteins and polypeptides. In the case of the nucleoside-derived components, in which we had a particular interest, the anomeric proton of the ribose occurs in an uncongested region of the spectrum between 5.9 and 6.2 ppm, while fucose methyl groups are fairly distinct at 1.2−1.4 ppm. Close inspection of the spectrum in these regions immediately suggested the presence of 10 or more ribonucleoside derivatives, some of which appeared to be fucosylated. NMR signals of the aromatic headgroups of the acylpolyamines, those of free polyamine chains, and those of citric acid are also easily discernible (Figure 2A).

For further characterization of this mixture, a phase-sensitive dqf-COSY spectrum was acquired. We found that this technique has significant advantages over the use of traditional magnitude-mode COSY or TOCSY spectra. The predictable antisymmetric shape of the cross-peaks and the embedded multiplicity patterns were especially helpful in distinguishing individual cross-peaks clearly from artifacts and each other, which, given the enormous degree of overlap, was of prime importance for the analysis. Furthermore, analysis of the cross-peak multiplicity patterns allowed for determining fairly accurate values for all coupling constants in the various proton spin systems. A small section of the dqf-COSY spectrum of the crude venom is shown in Figure 2B.

Starting with the anomeric protons of the ribose units around 6 ppm, signals representing the other ribose protons were identified in this dqf-COSY spectrum. The 0.5 ppm downfield shift of the signals of the methylene protons in the 5′ position of the ribose (which is consistent with that of HF-7) indicated some form of derivatization at this position. Because the dqf-COSY cross-peaks of these methylenes did not show any additional splitting as would be expected for a phosphorylated residue, we hypothesized that the 5′ position of the various riboses might be sulfated. This nicely exemplifies the usefulness of the dqf-COSY technique, which in this case allowed us to assess the multiplicity of the protons in position 5′ and thus to exclude 5′-phosphorylation, even though the corresponding signals are completely obscured in the one-dimensional spectra (Figure 2B).

It should be noted that while the relatively simple appearance of the ^{1}H NMR spectrum around 6 ppm made the initial

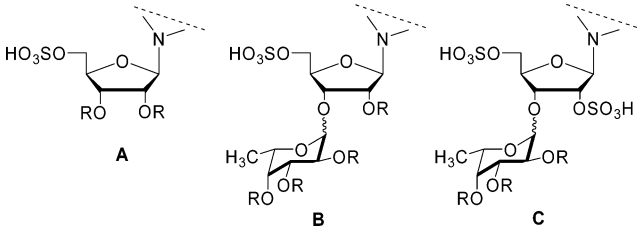


Figure 3. Partial structures of sulfated ribonucleosides in *T. agrestis* derived from NMR spectroscopic analyses of unfractionated venom. Two structures of type **A**, three structure of type **B**, and three structures of type **C** were detected.

detection of nucleoside derivatives particularly easy, the presence of nucleosides could have been detected just as well from the dqf-COSY spectrum alone had the ^{1}H spectrum been more crowded in this region.

In a similar fashion several fucose spin systems were identified from the dqf-COSY spectrum, working inward from the anomeric protons and the methyl groups. To determine some of the connectivity between the various carbohydrate spin systems, an (^{1}H,^{13}C)-HMBC spectrum of the mixture was acquired, using a variant of the HMBC sequence with improved resolution in F1 (vide infra). Using only NMR spectroscopy of the unfractionated venom for the initial analysis, we were able to propose partial structures for eight of the ten sulfated nucleosides present in the secretion (Figure 3).

In some cases we have found that initial NMR experiments of unfractionated materials provide enough information to determine the structures of all major components;[8] however, as evident from the spectra in Figure 2, the composition of the *T. agrestis* venom is extremely complex, necessitating that the sample be fractionated by reversed-phase HPLC. In the first chromatographic step applied to the native venom, the HPLC peaks tended to be fairly broad, possibly due to the aggregation of acidic and basic components. Thus, the venom was divided into early, middle, and late eluting fractions, with the two earlier eluting fractions containing nucleoside derivatives and the later eluting fraction containing polyamines and peptides. When, after evaporation of the solvent, we examined the contents of the first fraction, we expected to find a mixture of the most polar, bissulfated ribonucleosides that we had tentatively identified via analysis of the unfractionated venom (type **C** in Figure 3). However, what should have been several different compounds, turned out to be primarily monosulfated guanosine and free fucose, while the expected bissulfated ribonucleosides were absent. This was of immediate concern, since the initial NMR spectra did not indicate the presence of free fucose.

As pointed out earlier, spectra of the unfractionated natural secretion not only provide structural data, but also serve to document the original composition of the mixture. This is exemplified by our analysis of the bissulfated compounds. By comparing the results of our fractionation process with the original data of the crude secretion, we were able to determine that the bissulfated nucleoside derivatives were undergoing decomposition. While contained within the venom mixture, these compounds are buffered by polyamines, peptides, and inorganic salts. However, we found that when isolated in their pure forms, the bissulfated, glycosylated nucleosides are quite unstable and quickly decompose into monosulfated guanosine and fucose, which is not surprising given the pK_a of monoalkylated sulfuric acid derivatives. We were gratified to find, however, that the addition of a small amount of d_5-pyridine to each HPLC fraction preserved the bissulfated nucleoside derivatives as their d_5-pyridine salts.

To improve separation, we chose an isolation protocol involving reversed-phase HPLC with a 3.4 mM trifluoroacetic acid (TFA)/water and methanol gradient. This small amount of TFA is sufficient to protonate amino groups and reduce the affinity of the sulfates to the column material, without lowering the pH enough to break the glycosidic linkages or to induce partial loss of sulfate.[13] To prevent the molecules from decomposing upon concentration, the nucleoside-containing fractions were neutralized immediately after collection by the addition of appropriate amounts of pyridine-d_5 (as had been necessary to preserve the bissulfated compounds).[14] Fractions of interest were then reexamined by ^{1}H NMR and dqf-COSY, and the resulting spectra compared to the original data, which confirmed that there had been no noticeable degradation. To obtain ^{13}C data for the isolated compounds, we had to rely entirely on HMBC and HSQC experiments, because the amounts of material available were very small.

HMBC spectra of the unfractionated venom and of isolated fractions were particularly important in characterizing the *T. agrestis* nucleosides, allowing us to establish the connectivity between nucleic bases and ribose and the positions of the glycosidic linkages. It is rather difficult to achieve this with other techniques such as mass spectrometry, due to the molecules immediately fragmenting into their basic ring systems, which may not allow one to distinguish between several similar structures.[15] However, the need for HMBC spectra presented a significant problem in our analysis, because it is the least sensitive of the 2D NMR spectra required for structural assignment. Given that only small amounts of venom could be obtained, the number of compounds we were able to characterize was primarily limited by the sensitivity of our specific version of the general HMBC experiment. The use of a nongradient version of the HMBC sequence without evolution of (^{1}H,^{1}H) couplings during t_1 helped increase sensitivity and clear up spectra of mixtures in cases of overlap.[16]

Using this HMBC version, we were able to observe C−H correlations from the ribose to the fucose, and vice versa, thus establishing the connectivity of the sugars in compounds **2** and **4** (Figure 4). It is worthy of note that these compounds were found to be almost completely insoluble in aprotic NMR solvents (such as DMF-d_6 and DMSO-d_6), which derailed an attempt to infer the position of the glycosidic linkages by the absence or presence of hydroxyl protons on the ribose or fucose moieties.

NOESY experiments were used to assign the configuration of the hexoses in compounds **2** and **4**. NOEs observed for the axial protons corroborated our assignment of these 6-deoxy-hexoses as fucoses, which originally had been based on coupling constant data obtained from dqf-COSY spectra. Of importance

(13) Similar concentrations of acetic acid or ammonium hydroxide proved to be significantly less effective at improving the HPLC separation.
(14) This is contrary to the method used to analyze the venom of *Latrodectus menavodi*. After prolonged exposure to 0.13 M (1%) TFA, ESI-MS analysis revealed the presence of several *unfunctionalized* nucleosides. See: Horni, A.; Weickmann, D.; Hesse, M. *Toxicon* **2001**, *39*, 425−428.
(15) (a) Zaia, J. *Mass Spectrom. Rev.* **2004**, *23*, 161−227. (b) Von Seggern, C. E.; Moyer, S. C.; Cotter, R. J. *Anal. Chem.* **2003**, *75*, 3212−3218. (c) Harvey, D. H. *Mass Spectrom. Rev.* **1999**, *18*, 349−451.
(16) Summers, M. F.; Marzilli, L. G.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 4285−4294.

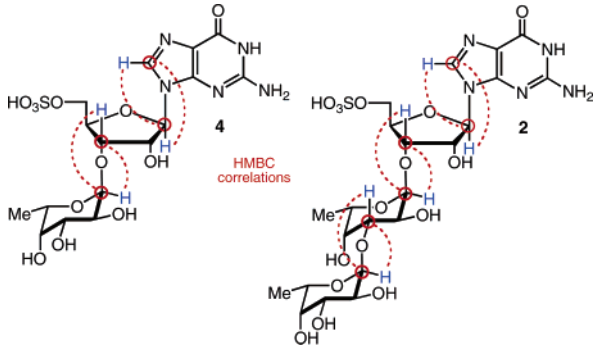


Figure 4. Select HMBC correlations used to determine the position of the glycosidic linkages in **2** and **4**.

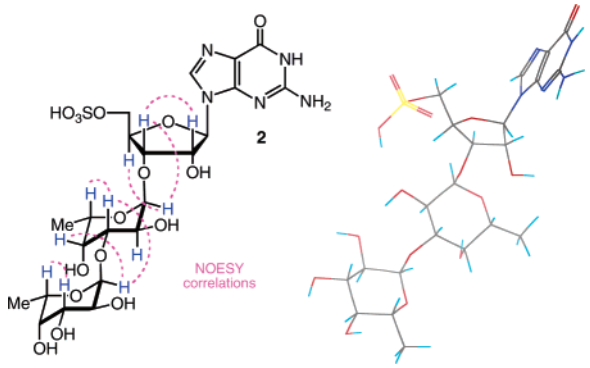


Figure 5. Select NOESY correlations and a molecular model of **2** which were used to assign the stereochemistry and connectivity of the sugars.

were also the NOEs between the protons in the 1 and 4 positions on the fucoses and the 2 and 3 protons of the ribose in compound **2** (Figure 5). These ribose−fucose and fucose−fucose correlations corroborated the proposed glycosidic linkages. A molecular mechanics model (Macromodel, Amber force field) of **2** confirms the validity of the observed NOEs, by demonstrating the proximity of the 1″ and the 3′ and 4′ protons as well as the 1‴ and the 3″ and 4″ protons.

As the last step in our analysis, we acquired mass spectra of the isolated compounds via negative-ion electrospray ionization. These MS data allowed us to confirm the presence of sulfate substituents and finalize the assignment of the various nucleic bases (Figure 6). UV spectra obtained during HPLC fractionation were consistent with the NMR- and MS-based assignment of the nucleic acid bases.

The nucleoside-containing fraction of *T. agrestis* venom represents about 50% (17 mg) of its total dry mass, the balance being made up of acylpolyamines, peptides, proteins, citrate, and inorganic salts. With the limited amount of *T. agrestis* venom available, we were able to completely characterize four sulfated ribonucleosides (**2**−**5**) and tentatively identify another three compounds (**7**−**9**), in addition to traces of HF-7 (**1**). For compounds **7**−**9**, we were unable to acquire sufficiently good HMBC spectra, because these compounds occur only at low concentrations in the venom.

The most abundant molecule in the entire venom is 5′-sulfated guanosine (**5**), which is found in approximately twice the molar concentration of that of all other nucleosides combined. From the NMR data obtained for the unfractionated secretion we know that **5** is actually present in the natural extract and is not a degradation product of the glycosylated or bissulfated compo-

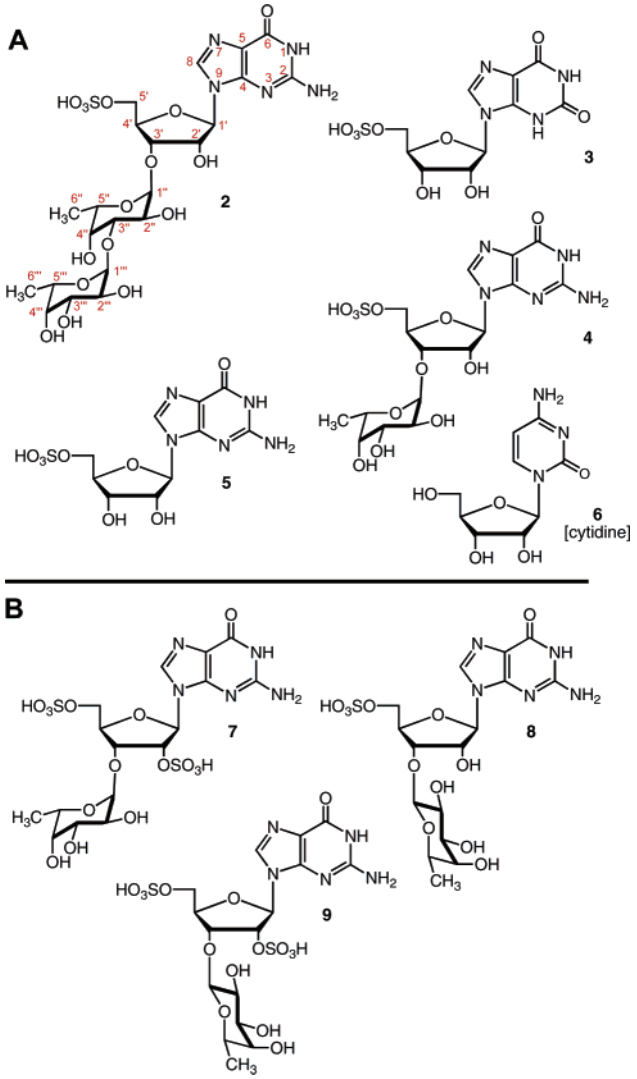


Figure 6. (A) New sulfated ribonucleosides **2**−**5** isolated and identified from *T. agrestis* venom in addition to nonsulfated cytidine (**6**). (B) Tentative structures **7**−**9** characterized on the basis of (^{1}H,^{1}H)-dqf-COSY, UV, and electrospray MS only.

nents. Most of the other ribonucleosides identified in the venom appear to be glycosylated derivatives of **5**. Compound **4** has an α-fucose in the 3′ position similar to HF-7 (**1**). Much like **4**, component **2** has a 3′-α-fucose to which is attached a second α-fucose. Interestingly, the second fucose moiety in **2** is attached in the 3″ position rather than the 4″ position, which is where the acyl group in HF-7 (**1**) is located. Compound **7** is a 2′,5′-bissulfated guanosine, again with an α-fucose at the 3′ position. Furthermore, the venom contains traces of the several 3′-*β*-fucosylated derivatives of **5**, most prominently the monosulfated **8**. The *β*-fucose linkage in **8** was inferred from the coupling constant of the anomeric fucose proton ($J_{1''-2''} = 8.3$ Hz), which is more than twice that of the same proton in **4** ($J_{1''-2''} = 4.0$ Hz).

Generally, the concentration of the guanosine derivatives in the venom decreases as more functionality is added to the basic 5′-sulfated core. In addition to the guanosines, we isolated and identified 5′-sulfated xanthosine (**3**), which in the venom is accompanied by very small amounts of corresponding fucosylated derivatives. Cytidine (**6**) is the only nonsulfated nucleoside we were able to detect in the venom.

Discussion

The development of improved methods for the discovery of biologically active natural products has been the subject of much discussion. We feel that there are at least two important issues that need to be addressed: (a) frequent disregard of the biological characteristics of the source organism and (b) a lack of control over the impact that extraction and fractionation procedures have on the biological material.

From a chemist's point of view, the second issue presents a serious challenge. It is the natural products chemist's bane that they usually know very little about the chemical properties of the compounds they are after. Choice of solvents, type of chromatography, and other fractionation conditions usually cannot be fine-tuned to the specific chemical properties of a new natural product simply because its structure has not been determined yet. As a result, a standard regimen of extraction and purification schemes has evolved, which is often applied without much regard to the nature of the extract. To what extent natural product extraction and fractionation schemes can skew analytical results has not been fully appreciated.

The present analysis of the venom of *T. agrestis* calls attention to the pitfalls of such a generalized approach to natural products discovery. A significant (and from a bioprospecting point of view certainly promising) family of compounds making up more than 50% of the material under investigation was lost using standard chromatographic techniques during our initial attempts to isolate the sulfated ribonucleosides in pure form. Reexamination of the venom of *H. curta*, the original source of the kainate inhibitor HF-7, using our direct NMR method immediately revealed the presence of at least five additional sulfated ribonucleosides, including several of the *T. agrestis* compounds, **2**−**5** and **7**−**9**.[17] In fact, we have found sulfated nucleosides in venoms of at least 12 of the 70 spider species we have recently investigated. Our conclusion is that NMR spectroscopic analyses of unfractionated materials represent a particularly valuable tool for finding new and interesting classes of secondary metabolites.

The difficulties encountered while characterizing these compounds lead us to believe that, in the past, the discovery of sulfated nucleosides (and glycosides) may have been hampered by their specific chemical properties. Because sulfated nucleosides do not ionize very well under electrospray conditions, their detection by mass spectrometry can present difficulty. The occurrence of sulfated nucleosides in nature might, therefore, not be limited to spider venom.

Surprisingly, a literature search revealed very little synthetic information about these relatively simple molecules,[18] and to our knowledge, biological properties of sulfated nucleosides such as **2**−**5** and **7**−**9** have not been evaluated.[19] These compounds are related to herbicidal 5′-sulfamoylnucleosides isolated from the bacterium *Streptomyces albus* (R 2374),[20] as well as to a family of phosphorylated nucleosides called adenophostins that affect calcium release.[21] Despite their structural simplicity, the sulfated nucleosides such as **2**−**5** and **7**−**9** may prove to be inhibitors of biological pathways involving phosphorylated nucleosides, in addition to their likely potential as neurotoxins. It would be particularly interesting to evaluate sulfated ribonucleosides, or corresponding deoxyribonucleoside derivatives, with regard to potential activity as antivirals or as inhibitors of cell cycle progression. Clearly, the amounts of venom components that can be isolated from *T. agrestis* will not be sufficient to investigate these possibilities, and neither would it seem feasible to obtain sufficient quantities of venom for broad activity-guided screening of the entire mixture. Therefore, syntheses and subsequent studies of biological activity remain important objectives.

Acknowledgment. We thank Professor Andrey Feodorov of Fauna Laboratories, Ltd., for providing the venom and the photograph of *T. agrestis*, the Cornell Institute for Research in Chemical Ecology (CIRCE), and the National Institutes of Health (Grant GM53850) for funding. The hospitality of the American Academy of Arts and Sciences to J.M., during the preparation of this paper, is acknowledged with pleasure.

Supporting Information Available: Analytical procedures and spectroscopic characterization of compounds **2**−**5** and **7**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA047416N

(17) These structures are currently being established by NMR spectroscopy.

(18) Holy, A. *Collect. Czech. Chem. Commun.* **1969**, *34*, 1261−1277.
(19) Surprisingly, the majority of the published biological assays for **5** concern its effects on taste and flavor enhancement: Kuninaka, A.; Kumagai, M.; Fujiyama, K.; Ogura, M.; Sakata, S.; Yonei, S. *Agric. Biol. Chem.* **1980**, *44*, 1437−1439.
(20) Gough, G. R.; Nobbs, D. M.; Middleton, J. C.; Penglis-Caredes, F.; Maguire, M. H. *J. Med. Chem.* **1978**, *21*, 520−525.
(21) (a) Jenkins, D. J.; Potter, B. V. L. *Carbohydr. Res.* **1996**, *287*, 169−182. (b) Hotoda, H.; Takahashi, M.; Tanzawa, K.; Takahashi, S.; Kaneko, M. *Tetrahedron Lett.* **1995**, *36*, 5037−5040.