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## Volume 5

## Structure Elucidation (Part B)

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# PREFACE

Natural products chemistry is one of the oldest branches of the chemical sciences, its origin dating back to the first decades of the 19th century, or even earlier. Presently, after almost 200 years of study, this discipline is still vibrant and evolving. What are the reasons for this continuous and continuing interest? Possible answers would have to include the challenges offered by the detection, isolation and purification procedures; by the permanently improving methods of structure elucidation; and by the complexities of the biogenetic pathways leading to these compounds.

However, one of the paramount reasons for pursuing natural products chemistry resides in the actual or potential pharmacological activity to be found in antibiotics, alkaloids, terpenoids, coumarins, sugars, flavonoids, lignans, sugars, or the like. To cite just a few numbers, one can estimate that the sum total of chemical compounds presently known is rapidly approaching the 10,000,000 mark. Of this very large figure, only about 50 have found clinical use as anti-cancer agents. What is especially significant is that of these approximately 50 compounds, around 35 are either natural products, derived from natural products, or else related in some ways to natural products. In other words, natural products and their derivatives exhibit a much greater propensity towards pharmacological activity than chemical compounds synthesized more or less at random in the laboratory.

The 22 chapters in the present book, edited by Prof. Atta-ur-Rahman, authored by some of the foremost natural product chemists around the world, and dealing with varied aspects of natural products chemistry with particular emphasis upon methods of structure elucidation, is a generous testimonial to the expanding dynamism of this realm of scientific endeavor.

Maurice Shamma
The Pennsylvania State University

## FOREWORD

The present volume is the fifth of this series, and the second covering the area of structure elucidation of new natural products. It covers the applications of modern spectroscopic techniques with particular reference to biologically important natural products including coumarinolignans, flavonoids, furanonaphthoquinones, antimalarials (eg. artemisinin), quassinoids, triterpenes, isoquinoline alkaloids, indole alkaloids, insect pheromones, polysaccharides from fungi and lichens and marine natural products. Other areas covered are those involving studies of carcinogenicity of estrogens, lignans biosynthesis, oligo (N-methylpyrrolecarboxamide) antibiotics, polyketide antibiotics, antitumor, antifungal and herbicidal antibiotics, sterols, carotane sesquiterpenes, sesquiterpene quinones, prostaglandin synthetase inhibitors and avian hemoglobins. The authoritative articles from leading natural product chemists should provide a wealth of informative material for research work in this field

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**June 1989** **Atta-ur-Rahman,** *Editor*

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## CONTENTS

# Structure Elucidation

# Proton and Carbon-13 NMR Assignments of Biologically Active Natural Products

**G. Blaskó and G. A. Cordell**

## 1. INTRODUCTION

Biologically active natural products obtained from medicinal plants are widely used in therapeutics and as biological tools. Due to the current substantial interest concerning the structure elucidation, biosynthesis, chemistry and metabolism of new, active natural products it is now standard practice to conduct detailed spectroscopic analysis of the isolates in order to assign unambiguously all the protons and carbons in their respective NMR spectra. Until recently, it would not have been reasonable to determine the complete proton and carbon assignments for a complex natural product. Now, the spectroscopic techniques are such that it is possible to **prove** these assignments without relying on prior information or using chemical shift correlation theory to assign carbon resonances.

In this chapter, the utilization of some recently developed NMR techniques will be presented on some selected natural products. The powerful combination of the correlation NMR spectroscopy methods (COSY, NOESY, HETCOR, HMQC and HMBC) with the one dimensional CSCM 1D and selective INEPT spectroscopic techniques will be demonstrated on natural products whose structure elucidation and/or rigorous, unambiguous spectral assignment was performed in our laboratory. The examples of natural products selected include compounds as diverse as coumarinolignans, naphthoquinones, flavonoids, dracaenones, sesquiterpenes, diterpenes, triterpenes, and pyrrolidine, indole, as well as isoquinoline alkaloids.

## 2. FT NMR METHODS

The introduction of pulse Fourier transform methods initiated a new era of NMR spectroscopy resulting in the intensive development of a plethora of new experimental techniques of crucial importance for the natural product chemist. Perhaps the most important single development is the ability to perform NMR correlation spectroscopy. Of the many available variations of this technique, three in particular have proven exceptionally useful in order to achieve unambiguous assignment of both the $^{1}H$- and $^{13}C$-NMR spectra of a new natural product, these techniques are i) homonuclear correlation spectroscopy ($^{1}H$-$^{1}H$ COSY), in which either geminal and vicinal or long-range couplings are emphasized, ii) two dimensional nuclear Overhauser experiments (nOe COSY or NOESY) in which proximate proton-proton relationships are displayed, and iii)

heteronuclear correlation spectroscopy ($^{1}H$-$^{13}C$ COSY or HETCOR) in which the correlation between a carbon and its attached proton(s) is displayed.

The latter technique, unfortunately, has three substantial disadvantages. First of all, HETCOR spectra typically afford no information, by definition, concerning the assignment of quaternary carbons. Secondly, significant amounts of material (at least 30 mg) are normally required, which may be impossible to obtain for a new natural product. Thirdly, the assignment of carbon signals which are very close ($\Delta\delta$ < 0.2 ppm) or which have identical chemical shifts is difficult because the digital resolution required can not be achieved. It became apparent that alternative techniques were needed in order to accomplish the unambiguous assignment of $^{1}H$- and $^{13}C$-NMR spectra of small quantitites of a compound. Several new pulse programming sequences (1-3) have recently been developed in order to achieve the complete assignment of complex NMR spectra. Two of these one-dimensional methods, the CSCM 1D (4) and the selective INEPT (5) techniques have proven extremely useful in our studies where the sample size was consistently a limiting factor.

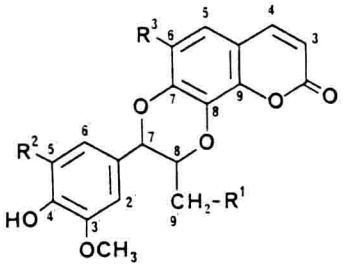
In the CSCM 1D technique (4) a carbon satellite either upfield or downfield of a proton is irradiated, magnetization is transferred to the attached carbon and a signal, either positive (upfield irradiation) or negative (downfield irradiation) is then observed. The experiment might therefore be interpreted as a one-dimensional technique corresponding to the $^{1}H$-$^{13}C$ HETCOR experiment. The major limitation to the technique is that it is not applicable in the case of potential proton overlap, but the substantial advantages of sensitivity and digital resolution outweigh this consideration. In the selective INEPT technique (5), the proton is irradiated with a soft proton pulse and the dwelling time prior to observation is varied depending on the anticipated coupling constant. Typically, the experiment is utilized for the selective enhancement of carbons three bonds away from the indicated proton where $J$ values in the range of 1-8 Hz are used. Judicious interpretation of selective INEPT experiments corresponds to a carbon-carbon connectivity study.

Among the new pulse programming sequences two additional double-quantum coherence NMR (2D INADEQUATE) experimens are worth mentioning as new effective tools for the natural product chemist. These are the sensitivity enhanced $^{1}H$-detected heteronuclear multiple-quantum coherence via direct coupling (HMQC) experiment (6-8) for protonated carbons, and via multiple-bond coupling NMR spectroscopy (HMBC) (9) for the determination of carbon connectivity bridging of heteroatom and nonprotonated carbons.

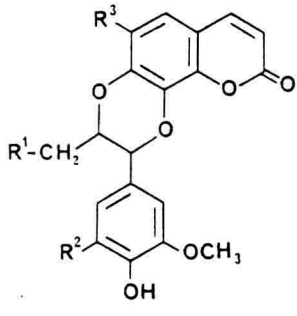
## 3. PROTON AND CARBON-13 NMR ASSIGNMENTS OF NATURAL PRODUCTS

### 3.1. COUMARINOLIGNANS

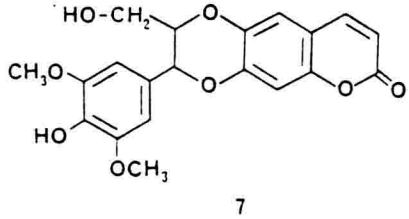
Coumarinolignans are a relatively new class of natural product, eight members (1-8) having thus far been isolated (10-19). A persistent problem in the structure elucidation of coumarinolignans, and for flavonolignans, xanthonolignans and certain neolognans is the unambiguous determination of the orientation of the substituents on the 1,4-dioxane nucleus. This is because there are two biogenetically possible couplings of an o-dihydroxy-coumarin with a phenylpropane moiety resulting in two isomers with the general structures **A** and **B**. Establishment of a method for the unequivocal differentation between structures **A** and **B** was made possible by the synthesis of sixteen coumarinolignans (20-22) including cleomiscosin A (1), daphneticin (2), propacin (3), aquillochin (4) and their respective positional isomers 5-8.



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 1 | OH | H | $OCH_3$ |
| 2 | OH | $OCH_3$ | H |
| 3 | H | H | $OCH_3$ |
| 4 | OH | $OCH_3$ | $OCH_3$ |



| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| 5 | OH | H | $OCH_3$ |
| 6 | OH | $OCH_3$ | $OCH_3$ |
| 11 | OH | $OCH_3$ | H |



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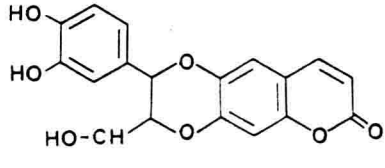

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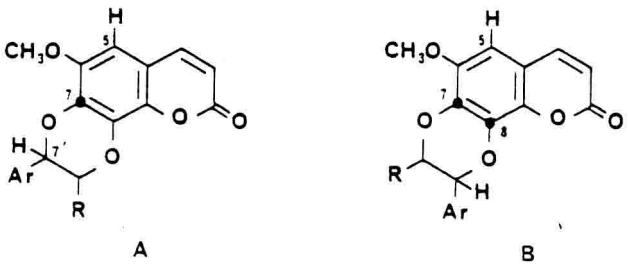
The strategy for the structure elucidation is shown conceptually in Scheme 1 and was described previously in our original work on cleomiscosin A (1) (15). If we consider the two possible regioisomers, **A** and **B**, and assume that the benzylic proton (7'-H) can be readily identified, then in isomer **A**, C-7 is one of the carbons expected to be three-

bond coupled, whereas in isomer **B**, C-8 should be coupled. The proton at C-5, however, should be coupled via three-bond coupling to C-7 (as well as C-8a and C-4). Thus the structural question centers on whether the same or different carbon atoms are found to be long range coupled when 5-H and 7'-H are irradiated.




Scheme 1. NMR Strategy for Distinguishing Between Coumarinolignan Isomers A and B

In order to establish the regiosubstitution on the dioxane ring of aquillochin (4), a series of selective INEPT experiments was performed on its diacetate derivative **9** using the strategy just described. According to Bax (5), the delays $\Delta_1$ and $\Delta_2$ should be determined by the long range coupling constant ($^{1r}J_{CH}$). In the case of a three bond C-H coupling constant, $^{3}J_{CH}$ can be estimated from the dihedral angle. Since the dihedral angles between C-7 and 7'-H and between C-6 and 8'-H are close to 90°, it was decided to use 1 Hz for calculation of the delay time. On the other hand, since the dihedral angle between 5-H and C-9, or between 5-H and C-7, is 180°, a maximum coupling constant would be expected, consequently, a $J$ value of 8 Hz was used in these experiments. Figure 1 shows a number of selective INEPT spectra of **9**. Irradiation of 5-H resulted in an increase of the two signals assigned to C-9 and C-7, together with a third less intense signal attributed to C-4. Enhancement of the C-6 signal indicated that 5-H was two-bond coupled to this carbon with a smaller coupling constant.

Irradiation of 7'-H significantly enhanced two carbon signals, namely C-7 and C-1', which indicated a connection between the aromatic substituent and C-7 through a dioxane ring oxygen. The enhancements of the aromatic carbon signals can be understood when the $J_{CH}$ values of a model compound such as toluene are compared (23). Of particular interest was the enhancement of C-4' which is five-bond coupled to 7'-H. Since the selective INEPT experiment is $J$ modulated, and the $^{5}J_{CH}$ value in toluene is 0.8 Hz, close to the value used in this experiment (1 Hz), it is not surprising to observe enhancement of a para-carbon by irradiation of the benzylic proton. For the purposes of comparison some of the relevant experiments with the other regioisomer will be discussed.

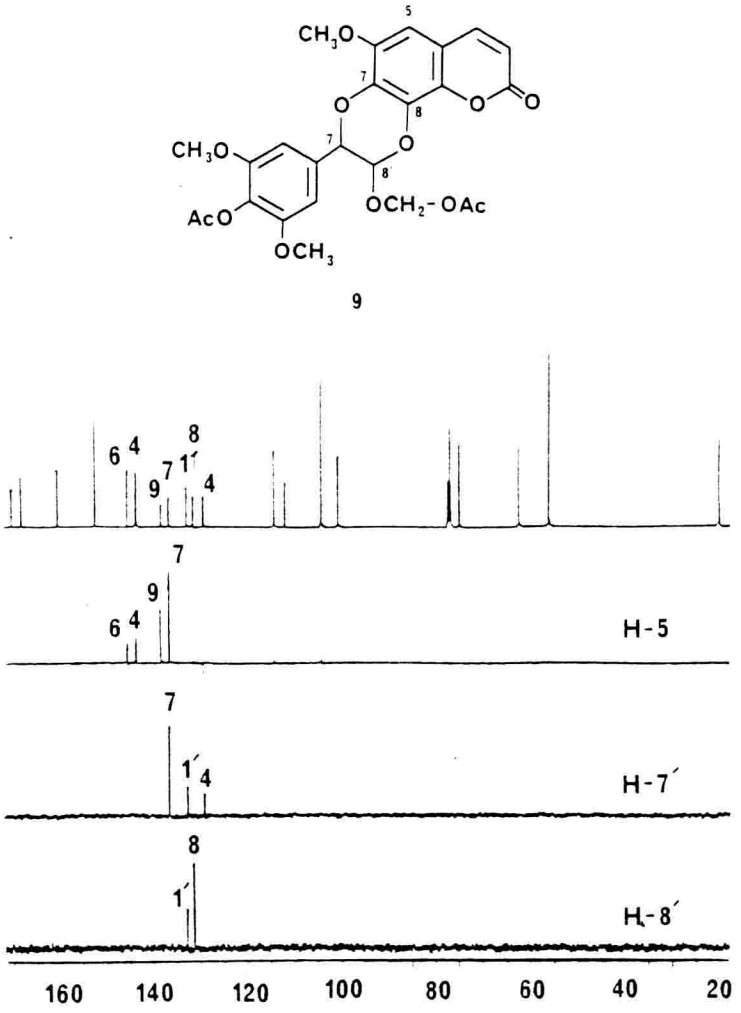



Figure 1. Selective INEPT Spectra of Aquillochin diacetate (9).

The $^{1}$H-NMR spectrum clearly indicated that it was the regional isomer, and the APT spectrum of aquillochin regioisomer diacetate 10 bore a strong resemblance to that of 9. Using the selective INEPT technique, irradiation of 5-H using a delay corresponding to a J value of 8 Hz resulted in the enhancement of three signals, namely C-9, C-7 and C-4, each of which is three-bond coupled to 5-H. On the other hand, irradiation of 7'-H and 8'-H provided very important information, as shown in Figure 2. Irradiation of 7'-H significantly enhanced the C-6 and C-1' signals, whereas irradiation of 8'-H resulted in the enhancement of C-7 and C-1', thereby demonstrating the relative positions of the substituents on the dioxane ring. Complete $^{1}$H- and $^{13}$C-NMR assignments of 9 and 10 are shown in Table 1.