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Structural studies of natural products by new NMR techniques

Haruo Seto

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

Abstract - It was found that a new 2D-NMR technique, HMBC (Heteronuclear Multiple Bond Connectivity) was an extremely useful technique for structural analysis of complicated molecules with many methyl groups such as polypropionate derived metabolites and terpenes. In addition, HMBC proved to be a method of choice for structural investigation of peptide substances. Its application for structural analysis or ^{13}C-NMR spectral assignments of erythromycin, lycoclavanol, capuramycin and complestatin is described in this paper.

INTRODUCTION

A new 2D-NMR technique HMBC (Heteronuclear Multiple Bond Connectivity)(ref. 1), which is very useful for structural studies of complicated natural products, has recently been proposed. This method detects ^{13}C-^{1}H long range couplings separated by two or three bonds ($^{2}J_{C-H}$ or $^{3}J_{C-H}$) by the most sensitive nucleus ^{1}H resulting in highly improved sensitivity. Since the information obtained by HMBC reveals the connectivity between proton spin systems separated by quaternary carbons or heteroatoms, and since its sensitivity is proportional to the signal intensity of proton signals, this method is extremely powerful for structural studies of complicated molecules with many methyl groups such as terpenes and polypropionate derived polyketides; in most cases, the signal intensity of sharp methyl proton resonances is an order of magnitude stronger than the other signals and their cross peaks can be detected with ease in the HMBC spectra. Another advantage of the application of HMBC for structural studies of complicated molecules with many methyl groups is that the cross peaks of methyl carbons can be very easy analyzed, since the carbon next to a methyl residue (i.e. $^{2}J_{C-H}$) is easily identified by analysis of H-H COSY and C-H COSY. In the case of terpene derivatives, many methyl carbons are linked to quaternary carbons. Thus, the distinction of $^{2}J_{C-H}$ and $^{3}J_{C-H}$, which is very difficult in most cases, can easily be accomplished.
HMBC can also be successfully utilized for detecting long range couplings between carbons and exchangeable protons. Therefore, it is a method of choice to reveal the amino acid sequences of peptide derivatives.
Application of this new NMR method for structural studies is described in this paper.

APPLICATION OF HMBC FOR STRUCTURAL STUDIES OF ERYTHROMYCIN

As a first example for structural studies by HMBC, the antibiotic erythromycin (Fig. 1) was selected as a model compound, since it possessed many methyl groups. In the HMBC spectrum of erythromycin (Fig. 1, in C_6D_6, only the methyl proton region is shown), ten C-methyl and one N-dimethyl groups show three or two cross peaks. Analysis of this spectrum could be made very straightforwardly. A methyl proton doublet at 1.27 ppm showed cross peaks with an ester carbonyl (C-1, observed as a fold back signal at ca. 58 ppm), a methine at 45.4 ppm (C-2) and an oxymethine at 80.7 ppm (C-3), which were accommodated in the partial structure, $-OOC_1-C_2H(CH_3)-C_3H(O)-$ by taking account of their ^{13}C chemical shifts.
Similarly, a methyl proton doublet at 1.53 ppm showed cross peaks with the oxymethine C-3 just explained, a methine at 40.1 ppm (C-4) and an oxymethine at 84.2 ppm (C-5), giving the partial structure, $-C_3H(O)-C_4H(CH_3)-C_5H(O)-$. Thus, the carbon skeleton from C-1 to C-5 was established. By repeating the same procedure, the connectivity of the carbon skeleton of the aglycone moiety of the antibiotic (C-1 to C-15) became clear. The connectivities $CH_2-C(CH_3)(OCH_3)-CH(O)-CH(O)-CH_3$ in cladinose, and $(CH_3)_2N-CH$ and $CH_2-CH(O)-CH_3$ in desosamine were also revealed by HMBC as shown in Fig. 1 (indicated by bold lines). In addition to these partial structures, detailed analysis of the HMBC spectrum made clear the remaining structures such as the linkages between C-3 and C-1", and C-5 and C-1' (data not shown) (ref. 2).

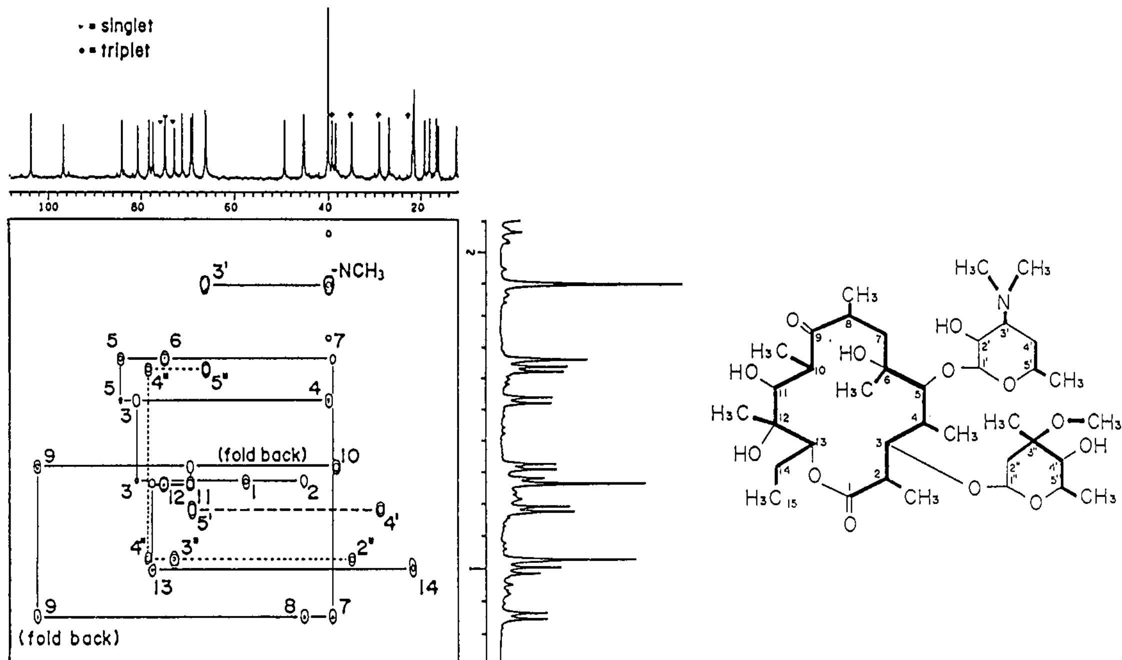

Fig. 1. HMBC spectrum of erythromycin

ASSIGNMENT OF THE ^{13}C-NMR SPECTRUM OF LYCOCLAVANOL

Lycoclavanol is a triterpene isolated from *Lycopodium clavatum* (ref. 3) and its structure had been determined by conventional methods. Its complete ^{13}C-NMR spectral assignment could be obtained by application of HMBC. As shown in Fig. 2, each methyl singlet displayed four cross peaks. For example, two methyl singlets at 1.17 ppm and 0.95 ppm showed cross peaks with the three common carbons (37.6 ppm, -C-, 43.8 ppm, -CH- and 75.2 ppm, -CH-O-). Thus, these three carbons were assigned to the carbons C-22, C-17 and C-21, respectively, and the methyl groups to C-29 and C-30. Likewise, the carbons connected by bold lines in Fig. 2 could be assigned very easily as tabulated in Table 1. The remaining methylene carbons could also be identified by detailed analysis of the HMBC spectrum (ref. 4).

It should be emphasized that the analyses of the HMBC spectra of compounds with many methyl groups such as erythromycin and lycoclavanol were very easily accomplished giving enough information to reveal the connectivity of the carbon skeletons.

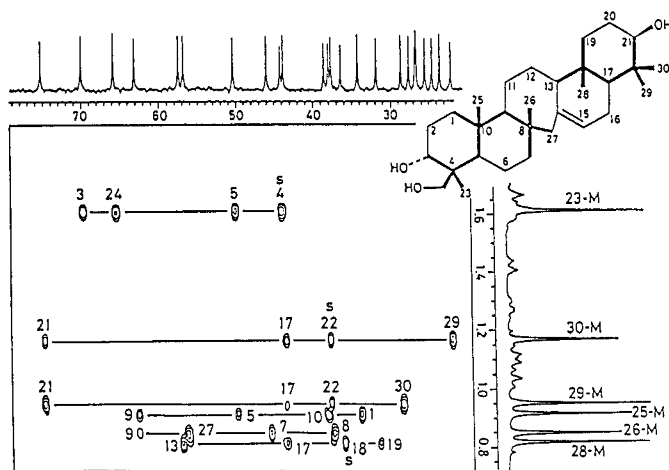

Fig. 2. HMBC spectrum of lycoclavanol

TABLE 1. C-13 NMR DATA OF LYCOCLAVANOL

No.			No.		
1	CH_2-	34.6	16	CH_2-	24.5
2	CH_2-	26.7	17	CH-	43.8
3	CH-	70.1	18	C-	36.4
4	C-	44.2	19	CH_2-	31.8
5	CH-	50.3	20	CH_2-	26.6
6	CH_2-	19.6	21	CH-	75.2
7	CH_2-	46.0	22	C-	37.6
8	C-	37.9	23	CH_3-	23.5
9	CH-	63.0	24	CH_2-	65.8
10	C-	38.5	25	CH_3-	16.6
11	CH_2-	25.7	26	CH_3-	20.1
12	CH_2-	27.6	27	CH_2-	56.8
13	CH-	57.4	28	CH_3-	13.7
14	C=	139.1	29	CH_3-	22.1
15	CH=	122.6	30	CH_3-	28.6

STRUCTURAL DETERMINATION OF A NEW NUCLEOSIDE ANTIBIOTIC, CAPURAMYCIN

Capuramycin ($C_{23}H_{31}O_{12}N_5$) is a new nucleoside antibiotic produced by Streptomyces griseus 446-S3 active against Streptococcus pneumoniae and Mycobacterium smegmatis ATCC 607 (ref. 5). The IR spectrum of I (KBr) showed the presence of -OH, -NH and amide functions (3400, 1680, 1515 cm^{-1}) and the absence of ester or carboxylic acid residues. Complete acid hydrolysis of I gave L-lysine and uracil.

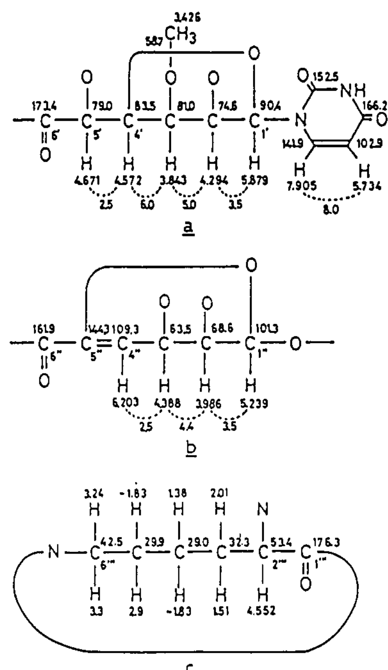

Fig. 3. Partial structures of capuramycin

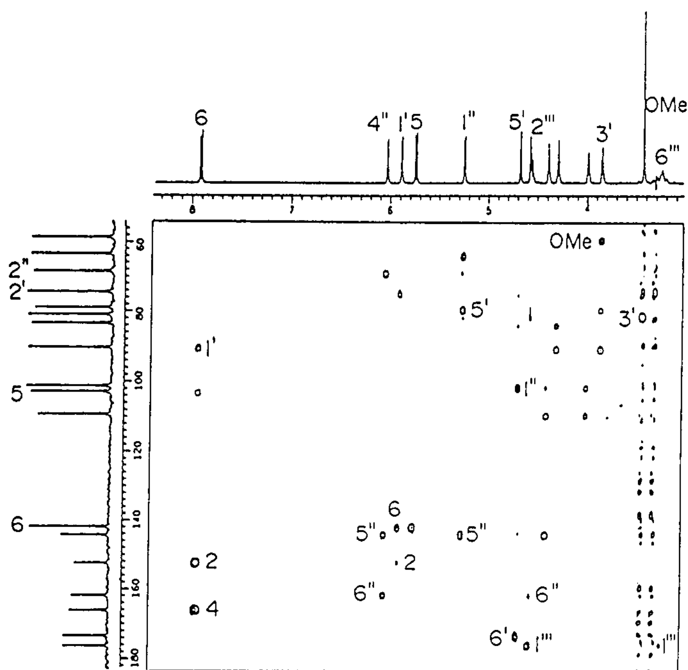

Fig. 4. HMBC spectrum of capuramycin

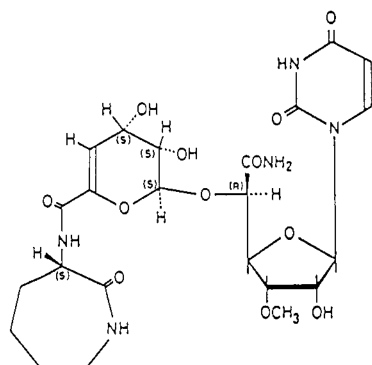

Fig. 5. Capuramycin

A detailed analysis of the COSY and ^{13}C-^{1}H COSY NMR spectra of capuramycin taken in CD_3OD showed the partial structures a, b, and c in Fig. 3. The relationships between protons and carbons not indicated by these methods were established by HMBC. Thus, the position of OCH_3 (δ_H 3.426) on C-3' was established by the cross peak observed between H-3' (δ_H 3.843) and OMe (δ_C 58.7) as shown in the HMBC spectrum of capuramycin (Fig. 4). The linkage of an amide carbonyl group to C-5' was confirmed by the cross peak between H-5' (δ_H 4.671) and C-6' (δ_C 173.4). In a similar way, the following connectivities proved by analyzing the same HMBC spectrum; H-1' to C-2 and C-6, H-1" to C-5" via an oxygen, H-3" to C-5", H-4" to C-5" and C-6", H-2"' to C-1"', and H-6"' to C-1"'.

The information on the linkage between these three fragments a-c was also obtained from the analysis of the HMBC spectrum. For example, H-5' (δ_H 4.671) and H-1" (δ_H 5.239) showed a cross peak with C-1" (δ_C 101.3) and C-5' (δ_C 79.0), respectively, to result in the linkage of fragment a and fragment b. The cross peak between H-2"' (δ_H 4.552) and C-6" (δ_C 161.9) attached fragment c to fragment b. Thus, based on HMBC spectral analysis, the planar structure of capuramycin was determined. Its absolute structure was established as shown in Fig. 5 by X-ray and ORD spectral analysis (ref. 6).

STRUCTURE OF COMPLESTATIN, AN INHIBITOR OF PROTEASE ACTIVITY OF COMPLEMENT IN THE HUMAN COMPLEMENT SYSTEM

HMBC can also be applied for detecting long range couplings between carbons and exchangeable protons. Its application for a peptide compound enabled to establish the total structure of complestatin. This metabolite isolated from the mycelia of Streptomyces lavendulae (ref. 7) strongly inhibits the hemolysis of sensitized erythrocytes by the complement system

(IC_{50}=0.1 μg/ml). It is the most potent compound among the known inhibitors with anti-complement activity. The molecular formula of complestatin was determined to be $C_{61}H_{45}O_{15}N_7Cl_6$ by elemental analysis and HR-FABMS spectral data.
The ^1H- and ^{13}C-NMR spectral data showed the presence of 1 X $-NCH_3$, 2 X CH_2, 6 X CH, 20 X -CH=, 24 X -C=, 1 X -COOH, 6 X -CO-NH- and 1 X -C=O.
Detailed analysis of the COSY spectrum of complestatin revealed the presence of the following units; 4 X -CO-NH-CH-, 1 X $-CO-NH-CH-CH_2-$, 1 x $-CH_2-CH-N-$, 2 X 1,4-disubstituted aromatic system and two ortho-coupled aromatic protons.

Analyses of the HMBC and H-H COSY spectra of complestatin established seven partial structures, i.e., six amino acid units and one fragment with a ketone function (A to F and G, respectively, in Fig. 6). The sequence of these fragments could also be obtained by analysis of long range couplings between methine and/or amide protons and the carbonyl carbon of the adjacent amino acid fragment. The arrows in the Figure indicate long range couplings between protons and carbons ($^2J_{C-H}$ or $^3J_{C-H}$). By taking into account the overlapping of the carbonyl carbons in Fig. 6 and additionally observed C-H long range couplings, the amino acid sequence of complestatin was established to give the total structure shown in Fig. 7 (ref. 8).

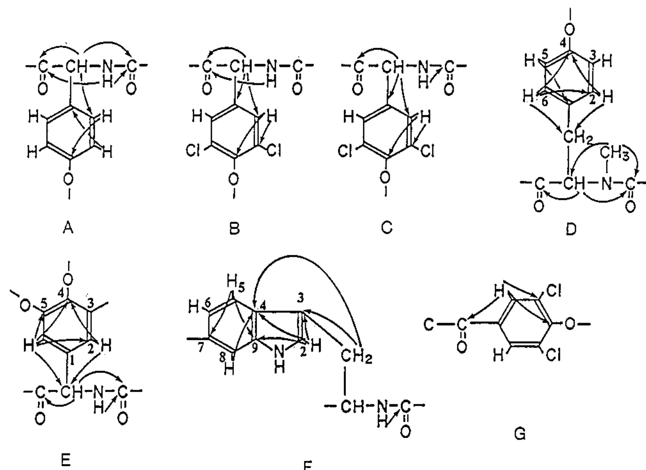

Fig. 6.
Partial structures of complestatin revealed by HMBC spectral analysis

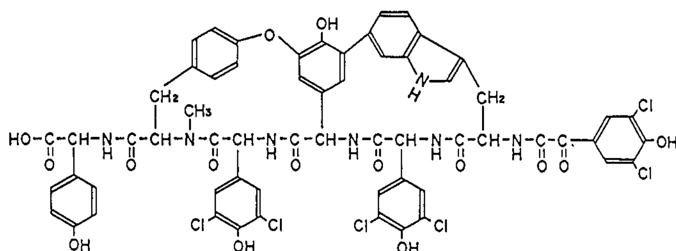

Fig. 7.
Structure of complestatin

Complestatin is structurally related to glycopeptide antibiotics. The main differences are that complestatin has no sugar units, and possesses an indole nucleus. It is interesting that the biological activities of complestatin and glycopeptide antibiotics are markedly different; the latter show very strong antibacterial activity to gram positive bacteria while complestatin inhibited the growth of a few gram positive bacteria at a very high concentration (ca. 2000 μg/ml). Tested so far, glycopeptide antibiotics showed no anti-complementary activity. These results suggest that difference of the biological activities of glycopeptide antibiotics and complestatin is not due to the presence of sugar units in the former group.

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