THE C-13 NMR SPECTRUM OF NOVOBIOCIN

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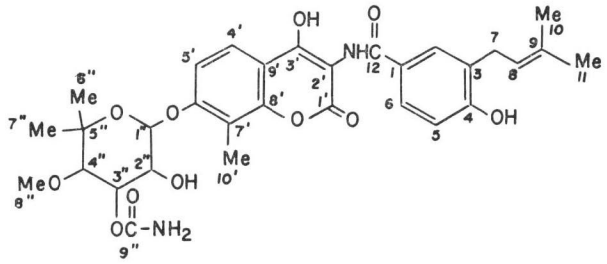
(Received for publication April 22, 1976)

The carbons of novobiocin have been assigned to peaks in the CMR spectrum on the basis of chemical shifts and off-resonance experiments on novobiocin and related compounds.

Novobiocin was the first isolated member of a family of antibiotics. Chlorobiocin[1] and coumermycin[2] were isolated at a later date and have not been used clinically. Since CMR spectroscopy has proved useful in biogenetic[3] and structural[4] work we now report our assignment of the C-13 spectrum of novobiocin. The CMR spectra of erythromycins,[5] rifamycins[6] and aminoglycosides[7] have been assigned by others.

Fig. 1. Novobiocin

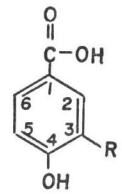


All spectra reported here were obtained on a Varian CFT-20. Multiplicities were determined using off-resonance techniques according to the manufacturer's instructions. For convenience, the carbons are numbered as in Fig. 1.

The anomeric, carbamyl, gem-dimethyl and methoxyl carbons (C-1″, 9″, 6″, 7″ and 8″) were easily assigned on the basis of chemical shift and multiplicity data (Table 1) on ethyl novioside[8] (**I**). Carbons 2″, 3″ and 4″ were assigned using a relationship between residual splittings and proton chemical shifts described by BIRDSALL.[9]

Four of the carbons in the isopentenyl side chain in 4-hydroxy-3-(3-methyl-2-butenyl)-benzoic

Fig. 2. Calculated *vs* observed shifts for **II**



	Shift	
Carbon #	Calculated	Observed
1	123.2	121.6
2	132.1	131.2
3	124.7	127.7
4	161.2	159.5
5	115.7	114.7
6	128.5	129.1

acid[8] (**II**) were assigned in analogy to 2-methyl-2-butene.[10] Furthermore, the doublet at δ 122.5 and the singlet at δ 131.9 disappear on hydrogenation over platinum.[8] The carbons in the benzene ring were assigned using the additivity of substituent effects[4] as shown in Fig. 2 (assuming R=methyl).

The carbons of the coumarin ring presented the most difficulty due to the seven quaternary carbons and their overlap with the carbons of the benzamido ring. We prepared novobiocic

Table 1. The C-13 chemical shifts for novobiocin and related compounds[1]

Carbon #	Novobiocin	I[2]	II	III[3]	IV	V[4]	VI[5]
1	123.6		121.6	123.7			
2	129.9d		131.2d	129.9d			
3	127.6		127.8	127.7			
4	158.8*		159.5	158.9			
5	114.6d		114.8d	114.6d			
6	127.6d		129.2d	127.5d			
7	28.5t		28.0t				
8	122.5d		122.5d	122.6d			
9	131.7		131.9	131.7			
10	17.7q		17.6q	17.7q			
11	25.5q		25.6q	25.6q			
12	167.0		167.8	167.1			
1′	158.2*			159.2	160.5	157.3	162.3
2′	101.9			101.0	100.9	124.1	91.4d
3′	160.7			161.0	161.3	128.5d	165.9
4′	121.9d			121.6d	121.9d	123.3d	
5′	110.3d			112.1d	110.3d	109.7d	
6′	157.1*			158.3	157.2	149.9	
7′	110.3			110.6	110.0	119.0d	
8′	150.4			151.2	150.8	151.0	153.8
9′	113.0			108.3	113.0	117.4	116.1
10′	8.3q			8.1q	8.3q		
1″	98.7d	100.9d			98.6d		
2″	69.0d	69.6d			69.0d		
3″	70.7d	70.9d			70.7d		
4″	81.1d	81.3d			81.0d		
5″	78.3	78.8			78.3		
6″	28.2q	28.3q**			28.5q**		
7″	22.8q	22.5q**			22.8q**		
8″	61.0q	61.0q			61.1q		
9″	156.5*	156.5			156.5		

* These four are indistinguishable from each other.

** Indistinguishable from each other.

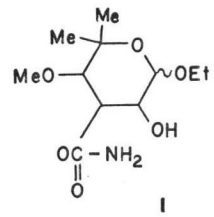
[1] The peaks are singlets except where labelled q, t or d for quartet, triplet and doublet. All spectra done on the Varian CFT-20 in d_6-DMSO. Shifts are given in parts per million relative to internal TMS. The multiplicities were determined using off-resonance experiments.

[2] Also, peaks at 62.3t and 14.8q for the ethoxyl carbons.

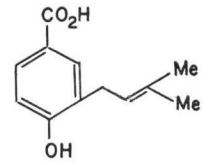
[3] Also, peaks at 155.7, 60.7t and 14.6q for the carbamyl and ethoxyl carbons.

[4] Also, peaks at 170.2, 168.9, 23.9q and 20.8 for the acetyl carbons.

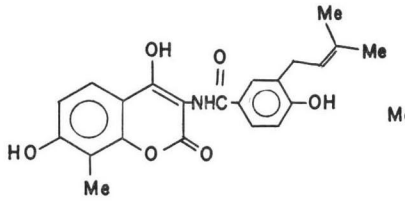
[5] Also, unassigned doublets at 132.5, 123.8, 123.4 and 116.4.



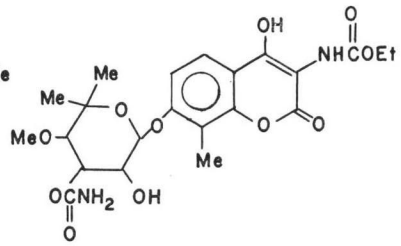
I



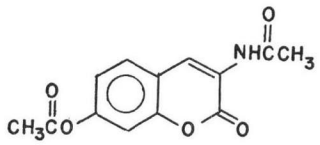
II



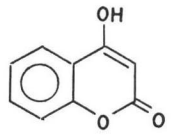
III



IV



V



VI

acid[8] (**III**), ethoxycarbonyl-novenamine[11] (**IV**) and 3-acetamido-7-acetoxycoumarin[12] (**V**) and purchased 4-hydroxycoumarin (**VI**) to facilitate the assignments. The CMR spectrum of coumarin has been published.[10]

The 10′ carbon is common to **III** and **IV** at δ 8.2. The farthest down-field singlets in **III** are the 1′, 3′, 6′ and 8′ carbons. The 3′ carbon was assigned to the peak at δ 161.0 due to its shift to δ 166 in the spectrum of novobiocin when sodium bicarbonate was added to the solution. The shifts for other carbons were considerably smaller. The 8′ carbon is assignable due to its consistent position in all the model compounds. We consider the 4, 1′, 6′, and 9″ carbons indistinguishable but have given them reasonable assignments in Table 2 based on the models. The assignment of C-2′ in **VI** was based on the expected upfield shift due to keto-enol tautomerism relative to its position in other models. Removal of this effect in **V** combined with amination results in a new singlet at δ 124.1. Reintroduction of the effect in **IV** results in an upfield shift of the C-2′ carbon relative to its shift in **V**.

The unassigned doublets in **VI** at δ132 and δ123 give rise to doublets at δ 119 and δ 109 in **V**. These must be due to the 5′ and 7′ carbons because of the expected upfield shift[4] of carbons *ortho* to an oxygen. One of the remaining two doublets in **VI** is due to C-4′ and so should be relatively unaffected by the further substitutions made. This can only be the doublet at δ 123. By difference the doublet at δ 116.4 is assignable to the 6′ carbon. This doublet disappears on oxygenation to give **V**.

Methylation of C-7′ should give rise to a new singlet 9 ppm downfield from its position in **V**. Only the doublet at δ 119 does this so we assign it to C-7′. Therefore, the peak at

δ 109 must be assigned to the 5′ carbon. The remaining singlet in **V** at δ 117 must be due to C-9′.

Acknowledgments

The author acknowledges useful discussions with K. RINEHART (University of Illinois) and S. MIZSAK, and the technical assistance of R. REID and D. SQUIRES.

References

1) DOLAK, L.: The structure of RP 18,631. J. Antibiotics 26: 121~125, 1973
2) KAWAGUCHI, H.; H. TSUKIURA & M. OKANISHI: Studies on coumermycin, a new antibiotic. I. Production, isolation and characterization of coumermycin A_1. J. Antibiotics, Ser. A 18:1~10, 1965
3) SEQUIN, U. & A. I. SCOTT: Carbon-13 as a label in biosynthetic studies. Science 186: 101~107, 1974
4) LEVY, G. C. & G. L. NELSON: Carbon-13 nuclear magnetic resonance for organic chemists. Wiley-Interscience, 1972
5) TERUI, Y.; K. TORI, K. NAGASHIMA & N. TSUJI: C-13 Nuclear magnetic resonance spectra of erythromycins. Tetrahedron Lett. 1975; 2583~2586, 1975
6) MARTINELLI, E.; R. J. WHITE & G. G. GALL: Carbon-13 NMR spectra of rifamycin. Tetrahedron 29: 3441~3448, 1973
7) YAMAOKA, Y.; T. USUI, H. SUGIYAMA & S. SETO: C-13 NMR spectra of some aminosugars and sugar-antibiotics, neomycin and kanamycin. Chem. Pharm. Bull. 22: 2196~2200, 1974
8) HINMAN, J. W.; E. L. CARON & H. HOEKSEMA: The structure of novobiocin. J. Am. Chem. Soc. 79: 3789~3800, 1957
9) BIRDSALL, B.; N. J. M. BIRDSALL & J. FEENEY: Simplified ^{13}C spectral assignments using a graphical method to present ^{13}C spectra recorded under conditions of proton off-resonance spin decoupling. Chem. Comm. 1972: 316~317, 1972
10) JOHNSON, L. E. & W. C. JANKOWSKI: Carbon-13 NMR spectra. Wiley-Interscience, 1972
11) DOLAK, L.: Process for N-acylating novenamine. U.S. Patent No. 3,890,297, June 17, 1975
12) RODIGHIERO, G. & C. ANTONELLO: Derivatives of 3-aminocoumarin and their antibacterial activity. Boll. Chim. Farm. 97: 592~601, 1958; Chem. Abstr. 53: 92101a, 1958