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Note

2D NMR Analysis of the polylactone derivative of colominic acid. Complete ^{1}H and ^{13}C NMR chemical shift assignments

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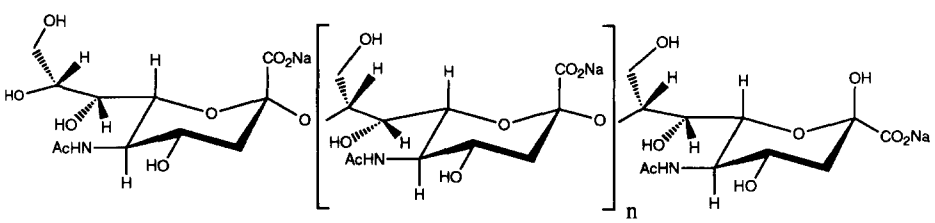
Colominic acid (**1**) is homologous to the weakly immunogenic group B capsular polysaccharide produced by *Neisserria meningitidis* that causes meningitis in humans [1].



colominic acid, sodium salt (**1**)

Solution-phase conformational studies of colominic acid suggest that the polysaccharide exists primarily as a random coil with localized helical regions that may serve as the antigenic determinant [2]. Lifely and co-workers have shown that, under acidic condi-

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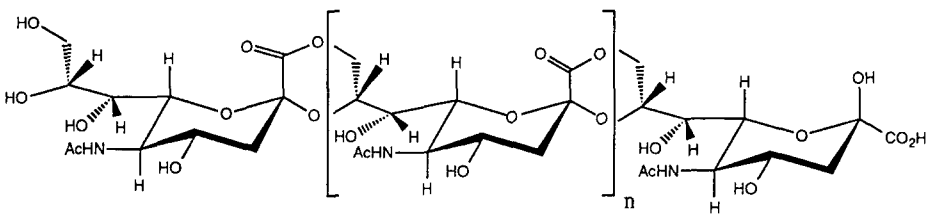
tions, colominic acid readily undergoes interresidue esterification, forming lactones between the carboxyl group and the adjacent C-9 hydroxyl (compound **2**) [3].



colominic acid polylactone (**2**)

Although complete ^{13}C NMR assignment of **2** was not reported, determination of the lactone regiochemistry was based upon a downfield shift of what was thought to be the C-9 resonance. In order to probe the conformational effects of polylactonization, we required complete ^{1}H and ^{13}C NMR assignments of **2**. During the course of our investigations, we discovered that the carbon resonances had been misassigned in earlier reports [3a]. Although the conclusion that lactonization occurs at the C-9 hydroxyl remains correct, the C-9 and C-4 assignments should be reversed. Reported herein is the first complete ^{1}H and ^{13}C assignment of the polylactone of colominic acid (**2**).

The full proton assignment of **2**, which until now had not been reported, became our first goal. The 1D ^{1}H spectrum (partially shown as a projection in Fig. 1) was acquired, and the amide N-H and C-3 protons were readily identified. Due to spectral overlap, 2D NMR analysis was required in order to assign the remaining peaks. Similarly the 1D ^{13}C spectra (partially shown as a projection in Fig. 1) clearly showed the carbonyl, anomeric, and acetamido resonances, but full assignment was not possible without 2D analysis. Identification of the two resonances occurring at approximately 69 ppm was particularly challenging. A 2D heteronuclear *J*-resolved (HET2DJ) experiment allowed us to identify two methylene protons occurring as triplets at 40.33 and 67.60 ppm, which were assigned to C-3 and C-9, respectively (Table 1). Next a HETCOR spectrum (using a $^{1}J_{C,H}$ value of 190 Hz) was obtained showing that the proton peaks occurring at 4.59 ppm and 4.47 ppm were coupled to C-9 (Fig. 1). A DQCOSY of **1** provided additional information, allowing most of the remaining assignments to be made. Although H-6 and H-7 cross peaks were not resolved, H-8 could be identified from cross peaks to H-9 and H-9′, and the remaining cross peak to H-8 allowed H-7 (3.51 ppm) to be identified. Having made the complete proton assignment, we were able to clearly identify all of the ^{13}C resonances except those belonging to C-7 and C-8. However, 1D traces of the HET2DJ combined with the DQCOSY data distinguished C-7 (occurring at 68.92 ppm) and C-8 (occurring at 69.33 ppm), Table 2.

The carbon resonances for C-4 and C-9 were previously reported as occurring at 69.8 and 68.1 ppm, respectively [3a]. The above set of experiments show unambiguously that this assignment should be reversed. In conclusion, 2D NMR analysis of colominic acid

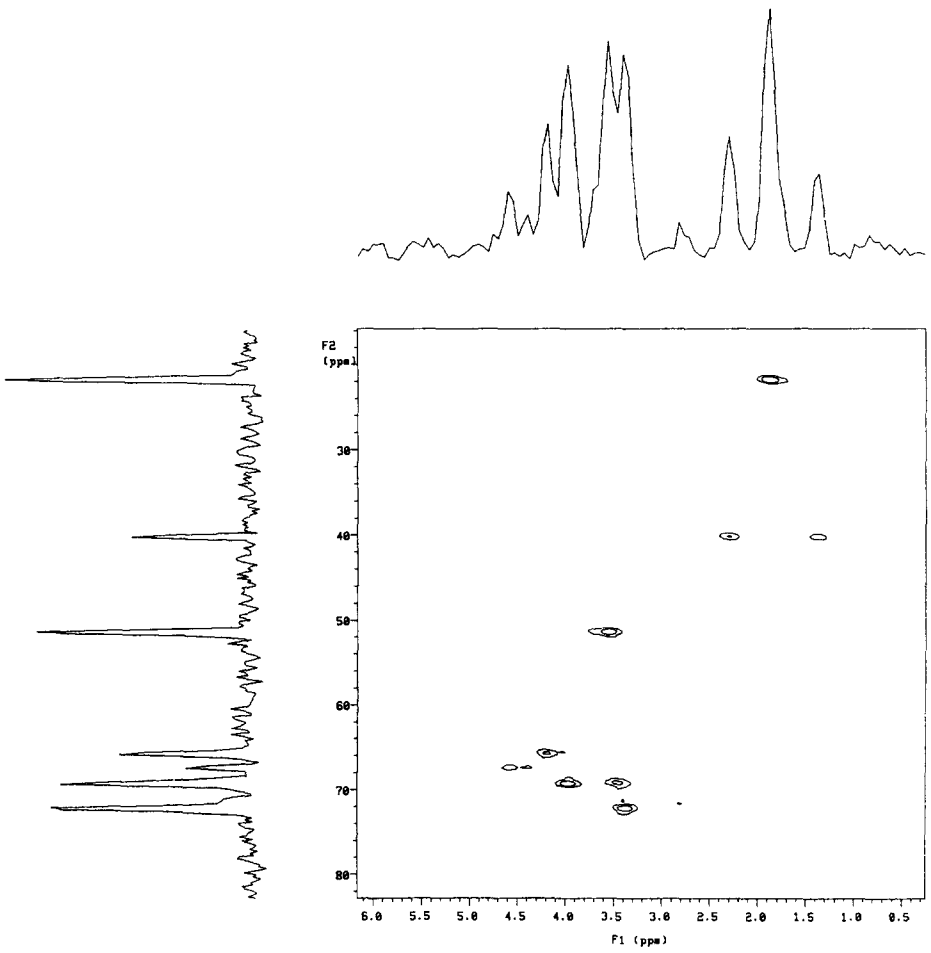


Fig. 1. 75.43-MHz HETCOR spectrum of colominic acid polylactone (**2**) (20 mg/mL in Me_2SO-d_6). The partial proton and carbon 1D spectra are shown as projections along the respective axes.

polylactone (**2**) allowed complete ^1H and ^{13}C assignment to be accomplished for the first time, providing important information for further conformational studies [4].

1. Materials and methods

Colominic acid (**2**) was purchased from Sigma Chemical Co. as the sodium salt. The polylactone **1** was prepared by reacting colominic acid with EDCI according to the literature procedure [3] and was dissolved in 1 mL of $(CD_3)_2SO$ for the NMR studies. All NMR spectra were obtained on a Varian Unity 300 spectrometer (for ^1H at 299.96 MHz and for ^{13}C at 75.43 MHz) at 97 °C. Chemical shifts are reported in ppm relative to the residual Me_2SO peak. The 2D double-quantum filtered homonuclear correlation

Table 1
Carbon multiplicity and $^{1}J_{C,H}$ coupling constant data from a 75.43-MHz HET2DJ spectrum of colominic acid polylactone (**2**) (20 mg/mL in Me_2SO-d_6)

Carbon peak (ppm)	Multiplicity	$^{1}J_{C,H}$ (Hz)
22.00	qt	117
40.33	t	166
51.59	d	158
65.94	d	175
67.60	t	189
68.92	d	190
69.33	d	162
72.37	d	158
95.92	s	0
165.02	s	0
171.88	s	0

(DQCOSY) spectrum was acquired as 2K × 2K data points with a spectral width of 4000 Hz. The data were processed with a phase-shifted sine bell and were zero-filled in the t_2 dimension. The 2D heteronuclear chemical shift correlation (HETCOR) spectrum was obtained as 512 × 2K data points. The ^{1}H spectral width was 4000 Hz and the ^{13}C spectral width was 18001.8 Hz. The data were processed with a phase-shifted sine bell in the t_1 and t_2 dimensions and were zero-filled in the t_1 dimension. The 2D heteronuclear (^{1}H and ^{13}C) *J*-resolved spectrum (HET2DJ) was acquired as 128 × 2K data points. The ^{1}H spectral width was 500 Hz and the ^{13}C spectral width was 18001.8 Hz. The data were processed with both a phase-shifted sine bell and a Gaussian window function in the t_1 and t_2 dimensions and were zero-filled in the t_1 dimension. Since decoupler gating was used, the actual *J* value is twice that of the measured *J* value ($J_{act} = 2 \times J_{obs}$).

Table 2
Complete ^{1}H and ^{13}C NMR chemical shift assignment of colominic acid polylactone (**2**)

Hydrogen atom	Chemical shift (ppm)	Carbon atom	Chemical shift (ppm)
H-3ax	1.39	C-1	165.03
H-3eq	2.29	C-2	95.72
H-4	4.20	C-3	40.33
H-4 (OH)	4.89	C-4	65.94
H-5	3.55	C-5	51.59
H-6	3.40	C-6	72.37
H-7	3.51	C-7	68.92
H-7 (OH)	5.14	C-8	69.33
H-8	3.99	C-9	67.60
H-9, 9′	4.47, 4.59	*N*-Ac (CH_3)	22.00
N-Ac (CH_3), *N*-H	1.91, 8.00	*N*-Ac (C=O)	171.88

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References

[1] D.L. Kasper, J.L. Winkelhake, W.D. Zollinger, B.L. Brandt, and M.S. Artenstein, *J. Immunol.*, 110 (1973) 262–268.

[2] J.C. Lindon, J.G. Vinter, M.R. Lifely, and C. Moreno, *Carbohydr. Res.*, 133 (1984) 59–74. R. Yamasaki, *Biochem. Biophys. Res. Commun.*, 154 (1988) 159–164. F. Michon, J.-R. Brisson, and H.J. Jennings, *Biochemistry*, 26 (1987) 8399–8405. R. Yamasaki and B. Bacon, *Biochemistry*, 30 (1991) 851–857. J.-R. Brisson, H. Baumann, A. Imberty, S. Pérez, and H.J. Jennings, *Biochemistry*, 31 (1992) 4996–5004.

[3] (a) M.R. Lifely, A.S. Gilbert, and C. Moreno, *Carbohydr. Res.*, 94 (1981) 193–203. (b) M.R. Lifely, A.S. Gilbert, and C. Moreno, *Carbohydr. Res.*, 134 (1984) 229–243.

[4] T.M. Flaherty and J. Gervay, unpublished work.