Complete Spectroscopic Structural Characterization of Novobiocin, Isonovobiocin, Decarbamylnovobiocin, 2"-(*O*-Carbamyl)novobiocin, and Novobiocin-2",3"-carbonate

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The rigorous structural characterization of novobiocin, is reported using mass spectrometric, infrared and nmr spectroscopic analysis. Complete nmr assignments are reported. Previous reports in the literature had left some quaternary carbon resonances unassigned. Isonovobiocin and decarbamylnovobiocin, although known in the literature for a number of years, have never been completely characterized. Mass spectrometric fragmentation pathways and complete ^1H and ^{13}C nmr assignments are reported for these congeners for the first time. The mass spectral fragmentation pathway and nmr assignments are also reported for 2"-(*O*-carbamyl)novobiocin although the nmr assignments at lower field were reported previously. The structural characterization of novobiocin-2",3"-carbonate observed in the Test Assay procedure used for Novobiocin is reported for the first time.

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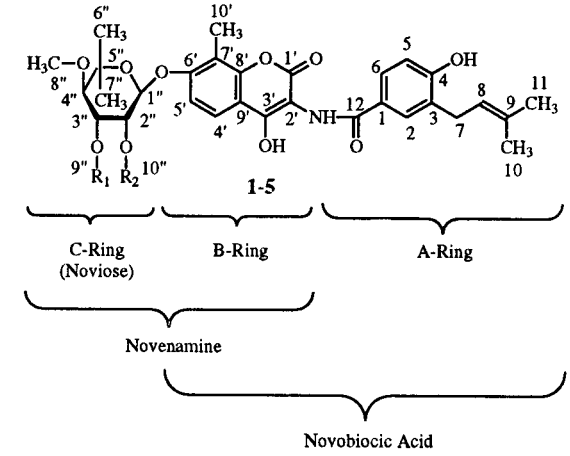
Introduction.

Novobiocin (**1**), a naturally occurring antibiotic, consists of a substituted benzoic acid moiety (A-ring) linked to coumarin (B-ring) *via* an amide bond; which is linked *via* a glycosidic bond to a noviose sugar (C-ring). Rings A and B without C are known as novobiocic acid and rings B and C are called novenamine. We are interested in characterizing the impurities and degradation products of the novobiocin molecule. An examination of the literature shows that although the structure of novobiocin and some of its degradation products and impurities have been known and discussed for some time, relatively few papers deal with the complete structural characterization of these molecules.

The independent synthesis of novobiocin was reported in 1963 [1], followed by reports of the formation of isonovobiocin (**2**) and decarbamylnovobiocin (**3**) in the fermentation process [2,3] and by chemical degradation [4]. The first mass spectrum of novobiocin where a molecular ion was observed was obtained by field desorption mass spectrometry [5]. The ^{13}C nmr spectrum was first assigned in 1976 [6] and later discussed along with the complete ^1H nmr spectrum [7,8]. Most recently, the complete ^1H and ^{13}C nmr and related two-dimensional experiments have been reported for novobiocin and for 2"-(*O*-carbamyl)novobiocin (**4**) [9]. Although the chromatographic separation of isonovobiocin (**2**) and decarbamylnovobiocin (**3**) has been reported [10], their detailed spectroscopic characterization has not.

In order to establish a database to facilitate the characterization of future degradation products and impurities of novobiocin (**1**), isonovobiocin (**2**), decarbamylnovobiocin (**3**), and 2"-(*O*-carbamyl)novobiocin (**4**) were isolated from fermentation produced novobiocin. An additional peak in the chromatogram, hitherto structurally uncharacterized, was also isolated and subsequently identified as novobiocin-2",3"-carbonate (**5**). These species were fully characterized by ^1H nmr, ^{13}C nmr, ir, and mass spectrometry.



Novobiocin (**1**): R_1 = -$CONH_2$, R_2 = -H
Isonovobiocin (**2**): R_1 = -H, R_2 = -$CONH_2$
Decarbamylnovobiocin (**3**): R_1 = -H, R_2 = -H
2"-(*O*-carbamyl)novobiocin (**4**): R_1 = -$CONH_2$, R_2 = -$CONH_2$
Novobiocin-2",3"-carbonate (**5**): R_1, R_2 =

Results and Discussion.

Novobiocin.

The mass spectrum of novobiocin (**1**) showed an intense protonated molecular ion, MH^+, at m/z = 613. Accurate mass measurement gave a molecular weight of

612.23256 daltons which matches the calculated accurate mass for the formula of $C_{31}H_{36}N_2O_{11}$ to 1.1 ppm. The ms/ms fragmentation of the protonated molecular ion does not provide any information about the structure of the noviose. The ms/ms spectrum is relatively simple and is summarized in Figure 1. The spectrum is dominated by a fragment resulting from the loss of the noviose sugar moiety and another fragment resulting from the loss of both the coumarin-derived (B-ring) and noviose (C-ring) portions of the molecule. This fragmentation provides little detailed structural information, but provides enough information to allow one to localize changes in structure of impurities or degradation products to either the A-, B-, or C-ring systems.

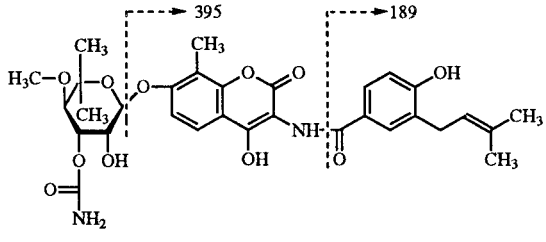


Figure 1. Mass spectral fragmentation of Novobiocin (1).

The nmr spectroscopy confirmed the structure of novobiocin (1), locating the carbamyl moiety at the 3"-position of the noviose subunit. Proton-proton connectivities were established by a homonuclear TOCSY spectrum; a GHSQC spectrum was employed to assign protonated carbon resonances after the proton-proton connectivity network was established. Long-range correlations from a GHMBC spectrum were then used to assign the quaternary carbons and to confirm the identity and integrity of the novobiocin carbon skeleton. Resonance assignments for novobiocin were consistent with those reported in the literature [6-8].

The infrared spectrum of novobiocin (1) is consistent with the reported structure. The differences between the spectrum of novobiocin (1) and the other compounds are discussed in the context of the individual compounds below.

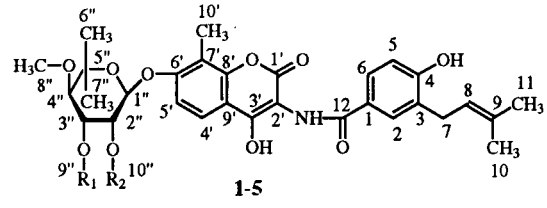
Isonovobiocin.

The mass spectrum of isonovobiocin (2) showed an intense protonated molecular ion, MH^+, at m/z = 613, which is the same as for novobiocin (1). Accurate mass measurement gave a molecular weight of 612.23161 daltons, which matches the calculated accurate mass for the formula of $C_{31}H_{36}N_2O_{11}$ to -0.49 ppm. The ms/ms fragmentation shows the same major fragments at m/z = 395 and 189, indicating that there are no formula changes to the coumarin-derived and A-ring portions of the molecule.

Carbamyl substitution was established at the 2"-position of the sugar residue based on the pattern of proton chemical shift perturbations and TOCSY couplings. In the case of the novobiocin parent, H2" and H3" resonate at 4.07 and 5.15 ppm, respectively. Carbamylation at the 2"-position would be expected to shift the H2" significantly downfield. Examining the ^1H chemical shift data contained in Table 1, we note that H2" is shifted as expected (4.07 → 4.93 ppm), while H3" experiences an upfield shift to 4.18 ppm. Carbon shifts (see Table 2), were consistent with 2-carbamylation. The other ^1H and ^{13}C resonance assignments corresponded favorably with those of the novobiocin (1) in this study and reported in the literature [6-9].

Table 1

Proton NMR Chemical Shift Assignments for Novobiocin (1), Isonovobiocin (2), Decarbamylnovobiocin (3), 2"-(*O*-carbamyl)-novobiocin (4), and Novobiocin-2",3"-carbonate (5)

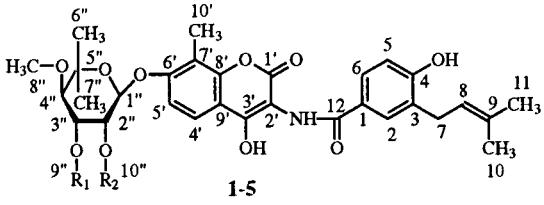


1-5

R_1	-CO-NH_2	H	H	CO-NH_2	R_1CO-R_2
R_2	H	CO-NH_2	H	CO-NH_2	
Position	1	2	3	4	5
2	7.74	7.74	7.73	7.77	7.74
4-OH	10.02	10.02	10.02	10.02	10.03
5	6.85	6.85	6.85	6.86	6.86
6	7.70	7.71	7.70	7.75	7.75
7	3.26	3.26	3.26	3.26	3.26
8	5.30	5.30	5.30	5.31	5.31
10	1.69	1.69	1.69	1.69	1.69
11	1.69	1.69	1.69	1.69	1.69
12-NH	9.20	9.20	9.19	9.21	9.22
3'-OH	11.97	11.98	11.96	12.00	12.05
4'	7.72	7.72	7.72	7.76	7.71
5'	7.15	7.18	7.16	7.18	7.2
10'	2.21	2.20	2.19	2.22	2.22
1"	5.52	5.59	5.52	5.62	6.09
2"	4.07	4.93	3.90	5.12	5.17
3"	5.15	4.18	4.01	5.28	5.14
4"	3.45	3.24	3.26	3.43	3.78
6"	1.04	1.07	1.01	1.11	1.12
7"	1.25	1.29	1.24	1.23	1.33
8"	3.46	3.50	3.49	3.48	3.49
9"	-NH_2 6.60	-OH 5.36	-OH 5.28	-NH_2 6.71	---
10"	-OH 5.56	-NH_2 6.68	-OH 5.00	-NH_2 6.71	---

Table 2

Carbon NMR Chemical Shift Assignments for Novobiocin (**1**), Isonovobiocin (**2**), Decarbamylnovobiocin (**3**), 2"-(*O*-carbamyl)-novobiocin (**4**), and Novobiocin-2",3"-carbonate (**5**)



1-5

R_1	-CO-NH_2	H	H	CO-NH_2	R_1-CO-R_2
R_2	H	CO-NH_2	H	CO-NH_2	
Position	**1**	**2**	**3**	**4**	**5**
1	124.2	124.2	124.3	124.2	124.2
2	129.8	129.8	130.2	129.9	129.9
3	127.2 [c]	127.2 [d]	127.6	127.4 [i]	127.3 [k]
4	158.2	158.2	158.7	158.4	158.4
5	114.2	114.2	144.4	114.3	114.3
6	127.4 [c]	127.3 [d]	127.6	127.3 [i]	121.4
7	28.0	28.0 [e]	28.1	28.1 [k]	28.1
8	122.5	122.5	122.7	121.9 [j]	122.6
9	131.4	131.4	131.7	131.5	131.6
10	17.7	17.7	17.8	17.8	17.8
11	25.6	25.5	25.6	25.6	25.6
12	160.6	160.6	159.6	160.61	166.2
1'	166.5	166.5	166.9	166.6	[a]
2'	101.4	101.4	101.5	101.7	101.9
3'	159.3 [b]	159.3 [b]	159.6	159.3 [g]	159.1 [b]
4'	121.8	121.8	121.9	122.6 [j]	127.5 [k]
5'	110.0	109.9	110.2 [f]	110.2 [h]	110.7
6'	156.9	156.8	157.3	156.7 [g]	156.4
7'	112.8	112.7	112.9	113.0	113.7
8'	150.6	150.5	150.9	150.6	150.6
9'	110.2	110.5	110.0 [f]	110.5 [h]	111.0
10'	8.3	8.2	8.4	8.3	8.4
1"	98.5	95.8	98.6	95.8	93.0
2"	68.7	72.3	67.8	70.0	76.1
2",3"-CO-	---	---	---	---	153.6
3"	70.3	65.7	70.9	69.8	77.6
4"	80.7	83.5	83.5	81.0	81.5
5"	78.1	78.0	78.1	78.3	76.4
6"	22.7	23.0	23.0	22.9	22.8
7"	28.4	28.2 [e]	28.7	29.0 [k]	27.0
8"	60.9	61.0	61.3	61.0	59.9
9"	156.2	---	---	155.7 [g]	---
10"	---	156.2	---	155.9 [g]	---

[a] Not observed; [b] Resonance is broad and poorly resloved; [c], [d], [e], [f], [g], [h], [i], [j] [k] Assignments may be interchanged.

The mid- and high frequency infrared data for novobiocin (**1**) and isonovobiocin (**2**) are very similar, with one exception. The N-H stretch at 3500 cm^{-1} seen for novobiocin (**1**) is shifted to 3200 cm^{-1} for isonovobiocin (**2**). N-H stretches at 3500 cm^{-1} are indicative of a N-H group relatively free of hydrogen bonding. Hydrogen bonding lowers the vibrational frequency of the N-H stretch. The N-H of isonovobiocin (**2**) at 3200 cm^{-1} must possess significant hydrogen bonding relative to novobiocin (**1**) and a different orientation of the carbamyl group. A weak vibrational peak at 1010 cm^{-1} in novobiocin (**1**) is shifted to 1033 cm^{-1} in isonovobiocin (**2**). This vibration is consistent with the C-O stretch of a carbamyl group. Based on the extreme similarity of the rest of the infrared data for novobiocin (**1**) and isonovobiocin (**2**), the carbamyl orientation is the only major difference between the two molecules.

Decarbamylnovobiocin.

The mass spectrum for decarbamylnovobiocin (**3**) showed an intense protonated molecular ion, MH^+, at m/z = 570, consistent with a molecule of novobiocin missing the carbamyl moiety. Accurate mass measurement gave a molecular weight of 569.22627 daltons, which matches the calculated accurate mass for the formula of $C_{30}H_{35}N_1O_{10}$ to 0.31 ppm. The ms/ms fragmentation shows the same major fragments at m/z = 395 and 189 (see Figure 1), indicating that there are no formula changes to the coumarin-derived, or A-ring, portions of the molecule.

The structure was confirmed by the nmr data *via* the absence of the two-proton NH_2 signal at 6.60 ppm seen in the ^1H spectrum of novobiocin (**1**), and the appearance of a new one proton signal at 5.28 ppm corresponding to the 3"-hydroxyl proton. Decarbamylation was also verified by slight upfield shifts of H2" (3.90 *vs.* 4.07 ppm in novobiocin (**1**) and H4" (3.26 *vs.* 3.45 ppm in novobiocin (**1**) resonances, and a pronounced upfield shift of H3" from 5.15 ppm for novobiocin (**1**) to 4.01 ppm in the decarbamyl analog (**3**). In the assigned carbon resonances, slight perturbations were seen in the C2" and C3" chemical shifts and a more pronounced, nearly 3 ppm downfield shift, was observed for C4". Most noteworthy was the absence of the carbamyl-carbonyl resonance that had been observed at 156.2 ppm in the ^{13}C spectrum of novobiocin (**1**). The balance of the ^1H and ^{13}C resonance assignments corresponded favorably with those of the novobiocin (**1**) in this study and reported in the literature [6-9].

The midfrequency infrared spectra of novobiocin (**1**) and decarbamylnovobiocin (**3**) are similar, suggesting that the bulk of the structures of the two molecules are comparable. One significant difference is the lack of the carbonyl stretching vibration of the carbamyl group at 1717 cm^{-1} in decarbamylnovobiocin (**2**). The N-H stretch of the carbamyl group at 3500 cm^{-1} in the parent molecule is also missing. The high frequency envelope centered at 3400 cm^{-1} for novobiocin (**1**) is broadened due to the presence of the -OH groups. The broadness of this envelope is enhanced in the spectrum of decarbamylnovobiocin (**3**), indicating the presence of additional -OH groups in the molecule relative to novobiocin (**1**).

2"-(*O*-Carbamyl)novobiocin.

The mass spectrum for the 2"-*O*-carbamylnovobiocin (**4**) showed an intense protonated molecular ion, MH^+ at m/z = 656, consistent with a molecule of novobiocin with an additional carbamyl moiety. Accurate mass measurement gave a molecular weight of 655.23673 daltons, matching the calculated accurate mass for the formula of $C_{32}H_{37}N_3O_{12}$ to -1.51 ppm. The ms/ms fragmentation shows the same major fragments at m/z = 395 and 189, indicating that there are again no formula changes to the coumarin-derived or A-ring portions of the molecule.

Carbamylation at both the 2"- and 3"-positions of **4** was readily confirmed from the proton nmr spectrum. Both H2" and H3" are shifted downfield relative to decarbamylnovobiocin (**3**), resonating at 5.12 and 5.28 ppm, respectively (also see Table 1). Perturbations of the noviose carbon shifts were also consistent with 2"/3" dicarbamylation. The ^{13}C nmr spectra verified the presence of the second carbamyl group. The limited sample, in conjunction with the numerous closely spaced carbon resonances, precluded the acquisition of GHMBC with both sufficient signal-to-noise and F_1 digital resolution to verify and assign the closely spaced resonant pairs. Hence, there are a number of pair-wise permutations possible in the ^{13}C resonance assignments as can be seen in Table 2.

The midfrequency infrared spectrum of 2"-*O*-carbamylnovobiocin (**4**) is very similar to novobiocin (**1**). The only differences are a significant increase in the intensity of the carbonyl stretch of the carbamyl group at 1717 cm^{-1} in the dicarbamyl species and the presence of the 1033 cm^{-1} vibration *plus* the analogous vibration for novobiocin (**1**) at 1010 cm^{-1}. Both of these differences in the spectrum of 2"-*O*-carbamylnovobiocin are consistent with the presence of a second extra carbamyl group on novobiocin. The presence of both the 1033 cm^{-1} C-O stretch and the 3200 cm^{-1} N-H stretch for 2"-O-carbamylnovobiocin indicate that the extra carbamyl group is in the *same* position as in isonovobiocin (**2**).

Novobiocin-2",3"-carbonate.

The mass spectrum for this molecule showed an intense protonated molecular ion at m/z = 596 indicating a molecular weight of 595 amu, 17 amu lower than that of novobiocin (**1**). The ms/ms fragmentation shows the same major fragments at m/z = 395 and 189, indicating that there are no changes in the empirical formulae of either the coumarin-derived or A-ring portions of the molecule. The "nitrogen rule" indicates that a molecule with a molecular weight of 595 amu must have an odd number of nitrogens. This makes the loss of an ammonia molecule, NH_3, the most likely explanation for the mass difference of 17 amu. Accurate mass measurement gave a molecular weight of 595.20217 daltons, fitting a molecular formula of $C_{31}H_{33}NO_{11}$ to -5.36 ppm. A structure consistent with the molecular formula and fragmentation could arise *via* loss of an NH_3 molecule in conjunction with cyclization to form a cyclic carbonate between two adjacent sugar hydroxyl substituents; *e.g.* at the 2"/3"-positions of the noviose unit. There have been no reports in the literature of any form of novobiocin cyclic carbonate.

The proton and carbon nmr spectra of the potential cyclic carbonate were examined for changes in the noviose sugar resonances, since the 3"-carbamyl group of novobiocin (**1**) is the only logical site from which ammonia could be lost. The carbamyl amino group, which resonates at 6.60 ppm in the proton spectrum of novobiocin (**1**), was absent from the proton spectrum of this isolate. The loss of ammonia presumes nucleophilic cyclization of the 2"-hydroxyl group with the 3"-carbamyl carbonyl to afford a 2",3"-cyclic carbonate, with ammonia lost in the cyclization process. Aside from the absence of the amino group, the next indication of modification of the sugar was afforded by the pronounced downfield shift of the anomeric H1" resonance from 5.52 ppm in novobiocin (**1**) to 6.09 ppm in the cyclic carbonate, the downfield shift consistent with modification of the 2"-hydroxyl to an "ester-like" structure. Utilizing the TOCSY spectrum, the H1" resonance was correlated to a multiplet at approximately 5.15 ppm which integrated for two protons. A further correlation was observed from the multiplet to a proton resonating at 3.78 ppm. To explain the proton spectrum, the downfield shift of H1" coupled with a downfield shift of the H2" resonance from 4.07 ppm in novobiocin (**1**) to approximately 5.15 ppm in the spectrum of the cyclic carbonate must involve modification of the 2"-hydroxyl group. Assuming the 3" proton to be essentially still "ester-like," it is logical to assume that the shift of H3" would be either unaffected or minimally affected by conversion of novobiocin (**1**) (5.15 ppm) to the corresponding cyclic carbonate **5**. This observation readily explains the two proton signals resonating at approximately 5.15 ppm. The closely similar shifts of H2" and H3" also accounts for the strongly coupled nature of the "multiplet" at *ca.* 5.15 ppm. Finally, the proton resonating at 3.78 ppm in the spectrum of the cyclic carbonate may be tentatively assigned as H4". Once again, relative to novobiocin (**1**), the H4" resonance is shifted downfield slightly from 3.45 ppm to the observed position at 3.78 ppm for the cyclic carbonate.

Examination of the GHSQC spectrum of the cyclic carbonate provided confirmatory evidence for the assumptions outlined above. First, the C1" resonance was shifted upfield nearly 5 ppm relative to novobiocin (**1**). This shift is consistent with the "esterification" at the 2"-position. The two proton multiplet at approximately 5.15 ppm was

associated with two carbon resonances at 76.1 and 77.6 ppm. Relative to C2" and C3" of novobiocin (**1**), which resonate at 68.7 and 70.3 ppm respectively, both of the resonances were shifted substantially downfield consistent with the proposed 2",3"-cyclic carbonate structure. More importantly, in the GHMBC long-range correlation spectrum optimized for both 6 and 10 Hz, long-range couplings were observed from the protons of the 5.15 ppm multiplet to a new quaternary carbon resonating at 153.6 ppm. That both of the protons of the "multiplet" couple to the 153.6 ppm carbon resonance supports the hypothesis that the carbonate linkage must be in cyclic form as proposed. Otherwise the H2" resonance would be unlikely to couple to the carbonyl resonance at 153.6 ppm across four bonds. All of the proton-carbon long-range couplings observed for the cyclic carbonate are summarized in Figure 2. Finally, the observed carbonyl shift of 153.6 ppm is also fully consistent with a cyclic carbonate structure.

The balance of the proton and carbon resonances of the cyclic carbonate **5** and novobiocin (**1**) are in excellent agreement with a single exception. The amide C12 carbonyl resonance in the cyclic carbonate resonates substantially downfield relative to the assigned resonance of novobiocin. Given that the assignment was confirmed by long-range correlation to the 12-amino proton resonance in both cases, the results are somewhat anomalous and difficult to rationalize, although both assignments are unequivocal.

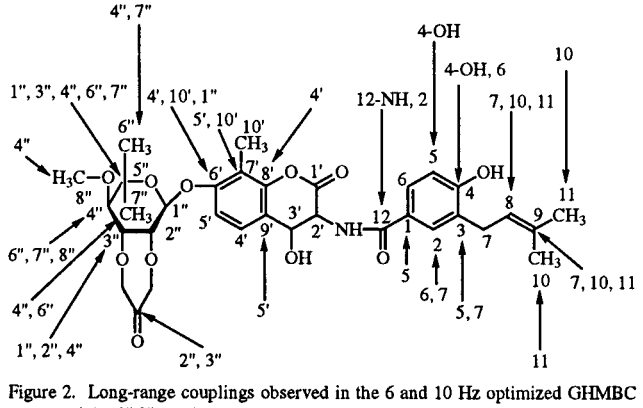


Figure 2. Long-range couplings observed in the 6 and 10 Hz optimized GHMBC spectra of the 2",3"-cyclic carbonate of novobiocin. Labels on arrows correspond to the protons which are long-range coupled to the carbon indicated by the arrow.

The infrared spectra of novobiocin (**1**) and the cyclic carbonate **5** are generally similar, suggesting that the bulk of the structures of the two molecules are comparable. One significant difference is the lack of the carbonyl stretching vibration of the carbamyl group at 1718 cm^{-1} in the cyclic carbonate spectrum. Both the symmetric (3352 cm^{-1}) and asymmetric (3490 cm^{-1}) N-H stretches of the carbamyl group for novobiocin (**1**) are also missing in the cyclic carbonate spectrum, confirming the lack of a carbamyl moiety in the structure. The presence of vibrations at 1817 cm^{-1} and 1161 cm^{-1} in the cyclic carbonate spectrum that are not present in the spectrum of novobiocin (**1**) are consistent with the C=O and C-O stretches, respectively, of a 5-membered cyclic carbonate group.

Conclusions.

The complete spectroscopic structural characterization of novobiocin (**1**), isonovobiocin (**2**), decarbamylnovobiocin (**3**), 2"-*O*-carbamylnovobiocin (**4**), and novobiocin-2",3"-carbonate (**5**) have been presented. ^1H and ^{13}C nmr data for novobiocin (**1**) and 2"-*O*-carbamylnovobiocin (**4**) agree with previously reported results [6-9] and serve as a reference for interpreting the spectra of the other less well characterized molecules. Although discussed often in the literature, isonovobiocin (**2**) and decarbamylnovobiocin (**3**) have not been characterized in nearly the detail as **1** and **4**. Complete nmr, ms and ir characterization are reported herein. A new molecule in the novobiocin family, novobiocin-2",3"-carbonate (**5**) is reported for the first time. The novobiocin-2",3"-carbonate (**5**) is completely characterized by nmr, ms and ir spectroscopy. The nmr data include ^{13}C and ^1H-results with long-range coupling results reported for the novobiocin-2",3"-carbonate (**5**).

EXPERIMENTAL

Sample Preparation and Isolation.

Novobiocin (**1**), its impurities and degradation products were detected by hplc using a 4.6 mm x 150, Jones Hypersil Silica (3 μ*M*) column with a mobile phase consisting of *n*-butyl chloride:methanol:tetrahydrofuran:acetic acid:water (902:35:30:30:3). An Altex 110A LC pump was employed at 1.0 ml/minute. Detection was at 280 nm using an LDC Spectromonitor D variable wavelength detector. Novobiocin (**1**) elutes in approximately 11-12 minutes. Relative retention times (rrt) for the other compounds are: novobiocin-2",3"-carbonate (**5**) rrt = 0.05; decarbamylnovobiocin (**3**) rrt = 0.4; isonovobiocin (**2**) rrt = 0.8; 2"-*O*-carbamylnovobiocin, rrt = 1.4. Samples for spectroscopic analysis were isolated and purified using a semi-preparative 22 x 250, mm Jones Apex Presil Silica (8 μ*M*) column on a Varex Versa Prep Preparative HPLC system with a mobile phase consisting of *n*-butyl chloride:methanol:trifluoroacetic acid (960:40:0.1). The flow rate was 40 ml/minute and detection was accomplished at 280 nm. Isolates were pooled, washed with milli-Q water to remove the trifluoroacetic acid, concentrated by rotary evaporation and placed in the vacuum centrifuge until dry.

Infrared Spectroscopy.

Samples for this study were prepared as potassium bromide pellets for infrared analyses. Data were collected from 4000-650 cm^{-1} H at 2-cm^{-1} resolution on a Nicolet 760 spectrometer equipped with a TGS detector or collected from 4000-400 cm^{-1} at 2-cm^{-1} resolution on a Nicolet 60-SX spectrometer equipped with a MCT-B detector. Sensitivity, expressed as instrument gain, was 1. The final spectra were the sum of 128 individual

scans. All data were processed as a Fourier transform utilizing a Happ-Genzel apodization function and were plotted as absorbance *vs.* frequency.

Mass Spectrometry.

Low resolution mass spectra were acquired on a Kratos MS-50RF or Finnigan MAT-900ST mass spectrometers operated at a resolution of approximately 1500 (10% valley definition). High resolution accurate mass measurements were performed on the same instruments operated at a resolution 12,000 while performing static magnet, E-scan peak matching. The ionization source was liquid secondary ion mass spectrometery (LSIMS) with a 19KV cesium ion beam on both the Kratos instrument and the Finnigan MAT instrument. The ms/ms studies were performed on a Finnigan TSQ-70 Triple Sector Quadrupole MS operated in the Fast Atom Bombardment (FAB) mode with *ca.* 6 kV Xenon atoms. The FAB produced protonated molecular ions were selected in Ql, fragmented in Q2 and the ms/ms daughter ion spectra recorded by scanning Q3. The ms/ms collision energy was 10 eV (instrument) using *ca.* 1 torr argon gas in the Q2 collision cell. In each case the sample was introduced into the instrument dissolved in a glycerol matrix.

Nuclear Magnetic Resonance Spectroscopy.

Isolates were dissolved in 99.992% dimethyl-d_6 sulfoxide (Cambridge Isotope Laboratories) or 99.996% dimethyl-d_6 sulfoxide (Isotec). The nmr spectra were acquired using Bruker AMX-400, AMX-500 or Varian INOVA 600 spectrometers operating at a ^1H observation frequency of 400.13, 500.13, or 599.75 MHz respectively. Both Bruker instruments were equipped with either a Nalorac 3 mm Z-axis gradient micro inverse probe or Bruker 5 mm Z-axis gradient inverse probe. The carbon spectra were acquired overnight using a Bruker AMX-500 operating at a carbon observation frequency of 125.77 MHz and equipped with either a Nalorac 3 mm micro dual probe or a Bruker 5 mm carbon dual probe. The Varian instrument was equipped with a Nalorac 3 mm gradient inverse triple resonance probe. Proton spectra were acquired using a standard spectral width of 14 ppm digitized with 16K points and zero-filled to 32K points. Homonuclear TOCSY spectra were acquired with mixing times ranging from 15 to 18.5 msec [11]. The data were acquired as 2K x 384 points and were linear predicted to 2K x 768 points and zero-filled to 8K x 8K prior to transformation. The GHSQC spectra were acquired as 1024 and 2048 x 128 points. Data were linear predicted in F_1 to 256 points, after which data sets were typically zero-filled to 4096 x 1024 points prior to Fourier transformation [12,13,14]. Delays based on the one-bond ($^1J_{CH}$) coupling constant were set for an assumed average coupling of 140 Hz. The interpulse delay was uniformly set to 1.0 sec. The GHMBC spectra were acquired as 2048 and 4096 x 256 or 384 points [15,16,17]. Data were uniformly linear predicted to 512 points in F_1 and then zero filled to either 4096 and 8192 x 1024 points prior to Fourier transformation. The low-pass J-filter was optimized for either 125 or 140 Hz; long-range delays were optimized for either 6 or 10 Hz (83 and 50 msec, respectively). Interpulse delays ranged from 1.0 to 1.8 seconds.

REFERENCES AND NOTES

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[1] B. P. Vaterlaus, K. Doebel, J. Kiss, A. I. Rachlin and H. Spiegelberg, *Experientia,* **19**, 383 (1963).

[2] V. B. Korchagin, V. V. Stepushkina and Z. E. Voinova, *Antibiotiki,* **11**, 107 (1966).

[3] I. V. Gracheva and V. A. Severina, *Antibiotiki,* **11**, 45 (1966).

[4] L. V. Birlova and D. M. Trakhtenberg, *Antibiotiki,* **11**, 395 (1966).

[5] K. L. Rinehart, J. C. Cook, K. H. Maurer and U. Rapp, *J. Antibiot.,* **27**, 1 (1974).

[6] L. A. Dolak, *J. Antibiot.,* **29**, 710 (1976).

[7] O. K. Sebek and L. A. Dolak, *J. Antibiot.,* **37**, 136 (1984).

[8] C. M. Pearce and J. K. M. Sanders, *Magn. Reson. Chem.,* **26**, 152 (1988).

[9] M. S. Kuo, D. A. Yurek, D. G. Chirby, J. I. Cialdella and V. P. Marshall, *J. Antibiot.,* **44**, 1096 (1991).

[10] K. Tsuji, P. D. Rahn and M. P. Kane, *J. Chromatog.,* **235**, 205 (1982).

[11] A. Bax and D. G. Davis, *J. Magn. Reson.,* **65**, 355 (1985).

[12] G. Bodenhausen and D. J. Ruben, *Chem. Phys. Letters,* **69**, 185, (1980).

[13] J. Ruiz-Cabello, G. W. Vuister, C. T. W. Moonen, P. VanGelderen, J. S. Cohen and P. C. M. VanZijl, *J. Magn. Reson.,* **100**, 282, 1992.

[14] A. Bax and S. S. Pochapsky, *J. Magn. Reson.,* **99**, 638 (1992).

[15] R. E. Hurd and B. K. John, *J. Magn. Reson.,* **91**, 648, (1991).

[16] R. E. Hurd and B. K. John, *J. Magn. Reson.,* **92**, 658, (1991).

[17] W. Wilker, D. Leibfritz, R. Kerssebaum and W. Bermel, *Magn. Reson. Chem.,* **31**, 287 (1993).