COLORADOCIN, AN ANTIBIOTIC FROM A NEW *ACTINOPLANES*

II. IDENTITY WITH LUMINAMICIN AND ELUCIDATION OF STRUCTURE

RONALD R. RASMUSSEN, MICHAEL H. SCHERR, DAVID N. WHITTERN, ALEX M. BUKO and JAMES B. MCALPINE*

Pharmaceutical Products Division, Department 47P, Abbott Laboratories, Abbott Park, Illinois 60064, U.S.A.

(Received for publication March 10, 1987)

Coloradocin was isolated from a fermentation broth by adsorption onto Amberlite XAD-2. The activity was eluted in MeOH and purified by gel filtration on Shephadex LH-20, followed by liquid-liquid chromatography on diol-bonded silica gel. The last two steps in the purification of this antibiotic included reverse-phase chromatography on C18-bonded silica gel and countercurrent chromatography on an Ito Coil Planet Centrifuge to give material of 90% purity. Analytically pure material was obtained by preparative HPLC. As a result of extensive homo and heteronuclear two-dimensional NMR studies, a structure was proposed for coloradocin. The structure consists of a decalin ring system fused to a 10-membered macrolactone which in turn is fused to a 14-membered macrolactone possessing an enol ether in conjugation with an unsaturated cyclic anhydride functionality. Coloradocin is related to a small class of antibiotics which include nodusmicin and nargenicin and was shown to be identical to luminamicin for which no structure has been reported.

In the preceding paper,[1] a new organism *Actinoplanes coloradoensis*, was shown to produce an anti-anaerobe antibiotic which was given the name coloradocin. The identity of coloradocin with luminamicin[2] and elucidation of structure is the subject of this paper. This work was presented in part at the Twenty-Sixth Interscience Conference on Antimicrobial Agents and Chemotherapy.[3]

Experimental

General Procedures

NMR spectra were obtained with either a General Electric GN 300 or GN 500 spectrometer using 5 mm probes. The various two-dimensional (2D) NMR techniques which were utilized are listed below along with the appropriate references: COSY[4] (2D ^{1}H-homonuclear *J*-correlation spectroscopy), CSCM[5] (2D ^{1}H-^{13}C chemical shift correlation map), FUCOUP[6] (fully-coupled ^{1}H-^{13}C heteronuclear correlation spectroscopy), CAMEL[7] (2D nuclear Overhauser effect (NOE)), and INADEQUATE[8] (2D ^{13}C-homonuclear *J*-correlation spectroscopy). Carbon multiplicities were determined by a DEPT[9] (distortionless enhancement of signals by polarization transfer) NMR spectrum. Fast atom bombardment (FAB) and electron impact (EI) mass spectra were determined on a Kratos MS-50 spectrometer. High resolution measurements were made at 10,000 resolving power. UV spectra were recorded with a Perkin-Elmer Lambda 3B UV/VIS spectrophotometer, IR spectra with a Nicolet 60 SX spectrophotometer and Raman spectra with a Cary 83 spectrophotometer. Optical rotations were measured in a 10-cm tube on a Perkin-Elmer model 241 polarimeter.

Isolation of Coloradocin (Assay Organism: *Bacteroides fragilis*)

Supernatant broth (49.8 liters) was adjusted to pH 4 and gently stirred with 2.5 kg of Amberlite XAD-2. The Amberlite XAD-2 was filtered off and washed first with 20 liters of H_2O which was discarded and then with 40 liters of MeOH which afforded 60 g of a dark brown oil. This material

was applied to a 4.5-liter bed volume of Sephadex LH-20 in MeOH and eluted with the same solvent. Active fractions were combined, resulting in the isolation of 19 g of a brown bioactive solid which was purified further in two successive low-pressure diol-LC[10] steps utilizing Sepralyte diol-bonded silica gel (40 μm). The first diol-LC column was run in biphasic CCl_4 - $CHCl_3$ - MeOH - H_2O (5:5:8:2) and the second in biphasic CCl_4 - MeOH - H_2O (5:4:1). The recovered material (4.7 g) was then chromatographed (under low-pressure) on 150 g of Sepralyte C18-bonded silica gel (40 μm) in a gradient going from 100% H_2O to 100% MeOH, giving 2.3 g of material (50% pure by ^1H NMR, $CDCl_3$). Coloradocin of 90% purity was obtained by countercurrent chromatography of 400 mg batches of 50% purity in an Ito Coil Planet Centrifuge[11,12] using the biphasic system $CHCl_3$ - MeOH - H_2O (1:1:1) with the lower (less polar phase) as the stationary phase. Material of 99% purity was obtained by semi-preparative HPLC.

Semi-preparative HPLC of Coloradocin

Semi-preparative HPLC was performed on a Perkin-Elmer system consisting of a Series 4 liquid chromatograph, LC-85 spectrophotometric detector with LC autocontrol (set at 344 nm) and a 3600-data station. A Regis Little Giant semi-preparative HPLC column (1×5 cm i.d.) packed with 3 μm C18-bonded silica gel was equilibrated in CH_3CN - H_2O (50:50) at a flow rate of 2 ml/minute. Up to 100 μl of 15 mg/ml of 90% pure coloradocin was injected each time and the peak at 5.3 minutes (99% pure) was collected. $[\alpha]_D^{29}$ +6.3° (*c* 1.0, CH_3CN); TLC (bioautograph, *Bacteroides fragilis*) Rf 0.86 ($CHCl_3$ - MeOH, 30:5, E. Merck Silica gel $60F_{254}$).

Characterization

UV spectra are shown in Figs. 1 and 2, IR and ^1H NMR spectra in Figs. 3 and 4, respectively. The UV spectrum of coloradocin taken in dry CH_3CN (Fig. 1, lower curve) changed when taken in CH_3CN - H_2O (Fig. 1, upper curve). After evaporation of the CH_3CN - H_2O and thoroughly drying the residue under reduced pressure, the UV spectrum was retaken in dry CH_3CN and found to be identical to the lower curve in Fig. 1. The UV curves in Fig. 2 reflect the behavior of a single sample of coloradocin in CH_3CN - H_2O at pH 7.0, pH 4.0, pH 11.0 and pH 1.0. After the sample sat at pH 1.0 for 2 hours the UV spectrum was again recorded and found to be identical to the lower curve in Fig. 1. The general instability of coloradocin in hydroxylic solvents was demonstrated further by its loss of *Bacteroides fragilis* activity upon exposure to hydroxide ion and also by changes in its ^1H NMR spectrum after standing in MeOH. On the basis of its UV spectrum, coloradocin was characterized as having a conjugated chromophore consisting of at least three sites of unsaturation (λ_{max}

Fig. 1. UV spectra of coloradocin.

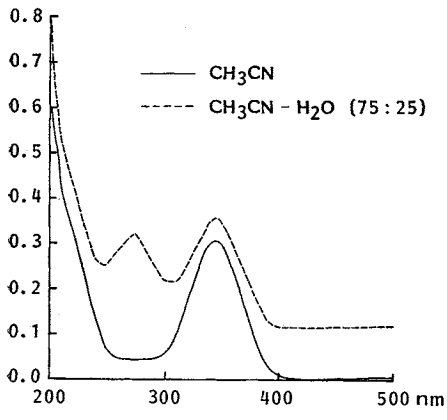


Fig. 2. UV spectra of coloradocin, CH_3CN - H_2O (75:25).

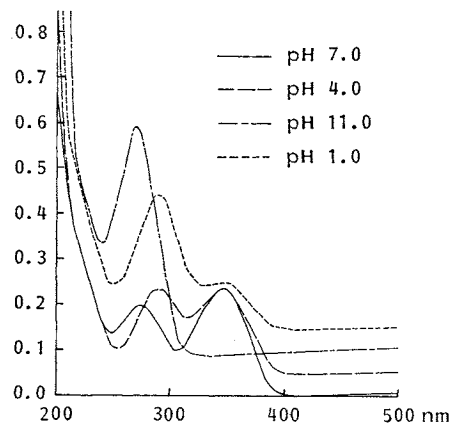


Fig. 3. IR spectrum of coloradocin (KBr pellet).

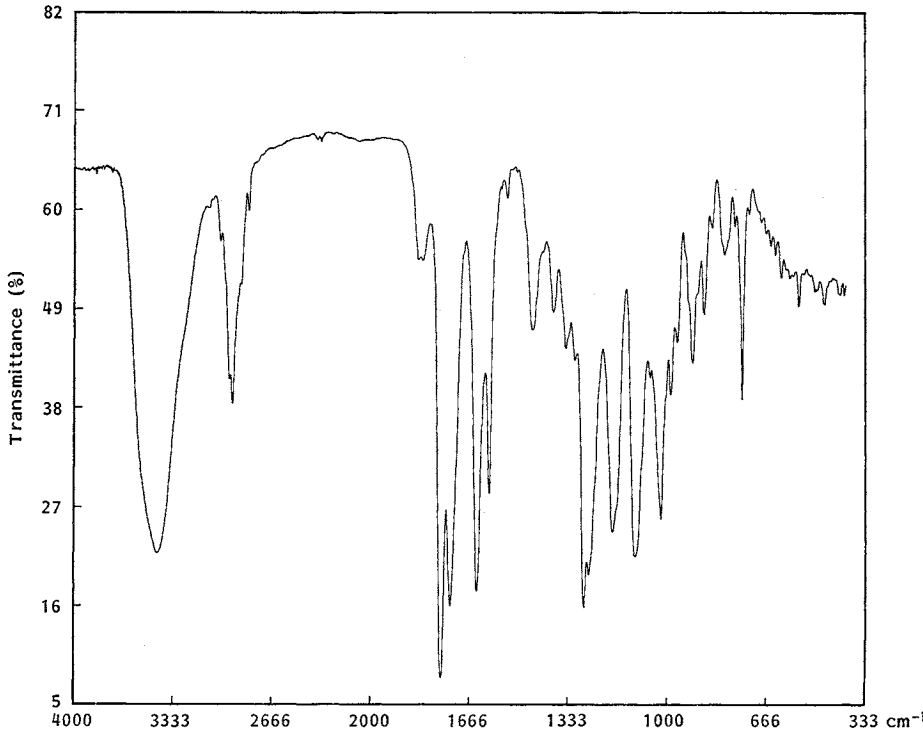
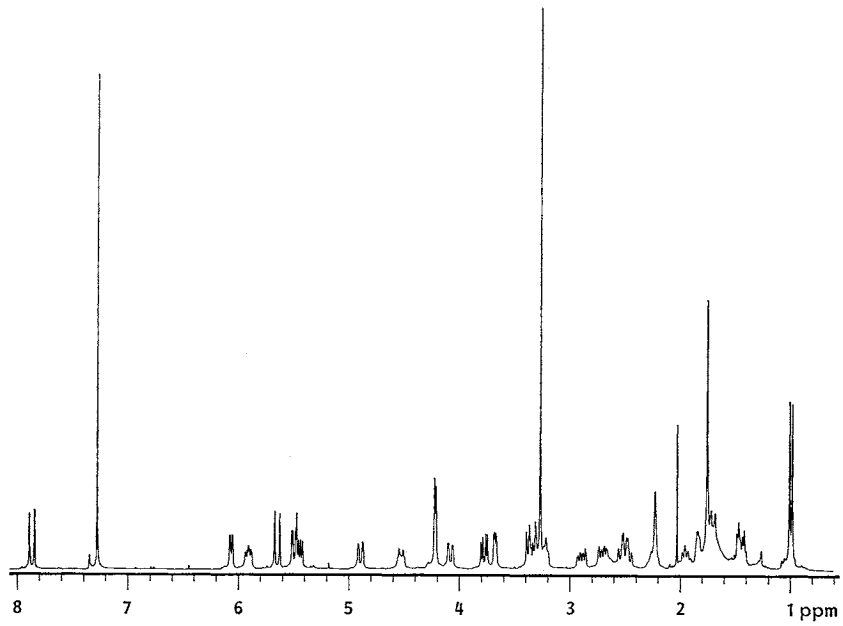


Fig. 4. ^1H NMR spectrum of coloradocin in $CDCl_3$ at 300 MHz.



344 nm). The IR spectrum (Fig. 3) of this antibiotic displayed two prominent ester type carbonyl bands at 1760 and 1730 cm^{-1}. Two other prominent bands at 1639 and 1599 cm^{-1} were most likely due to carbonyl or olefinic stretching. The ^1H NMR spectrum of coloradocin in $CDCl_3$ (Fig. 4)

Table 1. Comparison of coloradocin's MS, UV and IR spectral data with those of luminamicin.

	Coloradocin	Luminamicin
EI-MS (*m/z*)	614.2356[a]	614.236[a]
Molecular formula	$C_{32}H_{38}O_{12}$	$C_{32}H_{38}O_{12}$
UV λ_{max} nm (ε):		
CH_3CN	222 (sh), 344 (9,237)	—
Aqueous CH_3CN	222 (sh), 275 (3,278), 344 (7,151)	230 (sh), 291 (4,770), 347 (5,760)
Acid - aqueous CH_3CN	222 (sh), 292 (3,873), 344 (5,363)	300 (sh), 348 (7,280)
Alkaline - aqueous CH_3CN	271 (14,600)	271 (14,200)
IR (KBr) cm^{-1}	1835, 1760, 1730, 1640, 1600	1835,[b] 1760, 1730, 1640, 1600

[a] Theoretical: 614.2363 for $C_{32}H_{38}O_{12}$.
[b] Not shown in Table 1 of ref 2 but clearly present in the IR spectrum shown in Fig. 3 of same reference.
—: Not reported.

indicated the presence of a polarized double bond (protons at 7.86 and 5.65 ppm), as well as other protons on unsaturated centers (5.44 to 6.06 ppm). Several protons on carbons bearing oxygen resonated from 3.27 to 4.90 ppm. Several protons on saturated (less polar) carbons resonated from 0.99 to 2.87 ppm and included two methyl groups, one at 0.99 ppm and the other at 1.75 ppm. The resonance at 3.27 ppm was indicative of a methoxy group.

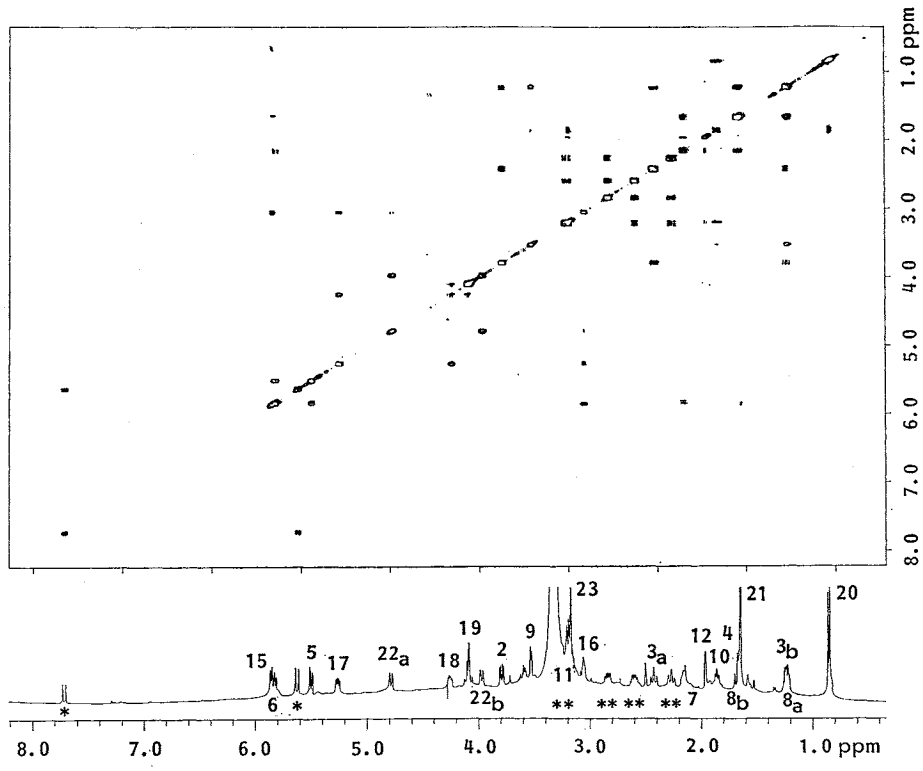
A comparison of coloradocin's MS, UV and IR spectral data with the corresponding data of luminamicin[2] (Table 1) established that these antibiotics were identical. The identity was further supported by the close correlation between the ^1H NMR spectrum of coloradocin ($CDCl_3$, Fig. 4) and that of luminamicin (acetone-d_6, Fig. 4 in ref 2). The interesting biological properties[1] of this unique microbial metabolite prompted us to determine it's structure.

Determination of Structure

A 2D *J* correlated map (COSY) and the accompanying ^1H NMR spectrum on the X-axis is shown in Fig. 5. The 6 spin-spin fragments determined by ^1H-^1H coupling (cross peaks in the COSY) are also shown in Fig. 5. The peak identifiers in Fig. 5 were chosen for correlation purposes. A DEPT ^{13}C NMR experiment (results not shown) revealed carbon multiplicities which aided in the interpretation of the CSCM shown in Fig. 6. The accompanying carbon and proton spectra are shown on the X and Y-axis respectively. Methylene carbons at 17.8 ppm (C-**), 32.9 ppm (C-3) and 65.0 ppm (C-22) failed to show cross peaks to protons in the CSCM whereas methylene carbon at 27.5 ppm (C-8) displayed a cross peak to H-8_a but not to H-8_b. However, when the data was replotted to obtain deeper contours (map not shown), cross peaks for the methylene carbon-proton correlations in question were found: 17.8 ppm (C-**) - 2.61 ppm (H-**), 3.37 ppm (H-**); 32.9 ppm (C-3) - 2.43 ppm (H-3_a), 1.22 ppm (H-3_b); 63.7 ppm (C-22) - 4.79 ppm (H-22_a), 3.97 (H-22_b); 27.5 ppm (C-8) - 1.21 ppm (H-8_a), 1.66 ppm (H-8_b). The types of carbons and connectivity results based on the COSY and CSCM are shown in Fig. 7. These results provided 6 structural fragments possessing the connections shown and an additional 8 disconnected carbon singlets. Part of the third fragment was further elaborated into a 6-membered ring.

Several more connections could be made after a FUCOUP NMR spectrum was obtained in $CDCl_3$. The connectivity map based on this long range ^{13}C-^1H coupling experiment is shown in Fig. 8. The previously disconnected carbon singlet at 141.5 ppm gave a cross peak (in the FUCOUP) with the

Fig. 5. 2D *J* correlated (COSY) NMR spectrum (500 MHz) of coloradocin in DMSO-d_6.
Fragments determined by ^{1}H-^{1}H coupling: 2 through 12, 20; 15 through 19, 22; 21-CH_3; 23-OCH_3; spin system*; spin system**.



proton on carbon 16 placing a double bond at this juncture in the second fragment. The carbon at 75.9 ppm was now coupled to the protons on carbons 4, 11 and 12. This revealed the presence of a second 6-membered ring fused to the first through carbons 7 and 12, forming a decalin ring system. The carbon at 75.9 ppm was also coupled to the proton on carbon 15. This provided a connectivity between the second and third fragments. The first and second fragments could be connected upon observing a cross peak for the sp^2 carbon at 155.6 ppm and the protons on carbon 19. Cross peaks for the carbon at 172.8 ppm, which had the chemical shift of an ester carbonyl, and H-3_b and H-17 allowed us to formulate a 10-membered macrolactone bridged to the decalin ring system through C-4 and the carbon at 75.9 ppm. The position of the methyl group (C-21) was decided by cross peaks for the methyl protons and the carbons at 75.9 ppm and 141.5 ppm. Likewise, the position of attachment of the methoxy group (C-23) was determined by long range coupling between the methoxy protons and the carbon at 81.8 ppm (C-2). The location of the two hydroxyl groups shown in Fig. 8 was decided by ^{13}C NMR difference spectra of coloradocin in $CDCl_3$ and in $CDCl_3$ - D_2O. Proton-deuterium effects on ^{13}C chemical shifts resulted in changes of Δ 0.2 ppm and Δ 0.1 ppm for C-11 and C-18 respectively. Additional long range ^{13}C-^{1}H couplings (FUCOUP) between the remaining disconnected carbon singlets shown in Fig. 8 and the ethylene moiety, as well as with the enol ether side chain gave equivocal connectivities. We were able, however, to relate the structural features of coloradocin, represented by the incomplete connectivity map in Fig. 8, to a small class of macrolide-like[13] antibiotics whose members are nodusmicin[14] and nargenicin.[15] These structural similarities are

Fig. 6. Chemical shift correlation map (CSCM) NMR spectrum (500 MHz) of coloradocin in DMSO-d_6. Imp.: Impurity.

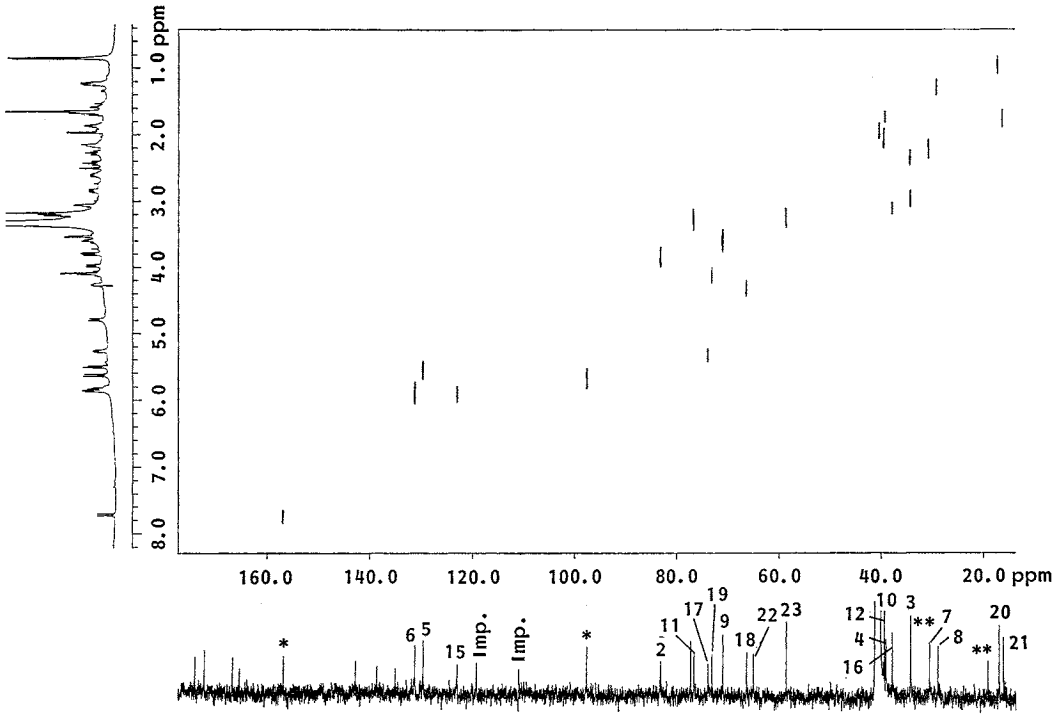


Fig. 7. COSY and CSCM connectivity results.

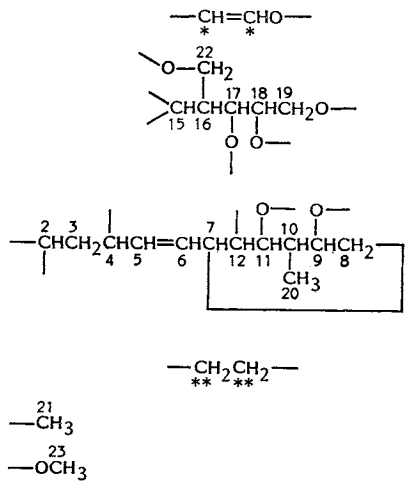
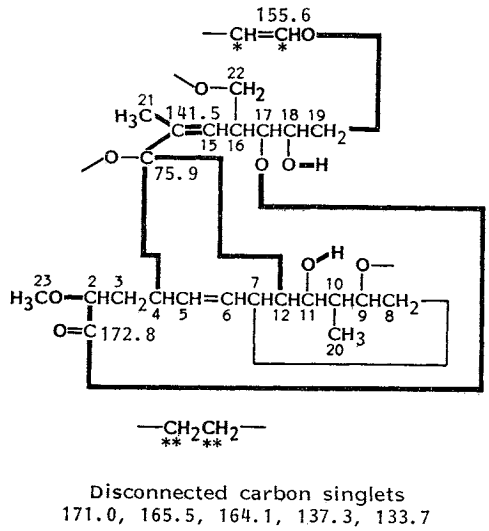


Fig. 8. FUCOUP ($CDCl_3$†) and ^{13}C NMR ($CDCl_3$-D_2O).

—: Difference connectivities.

† Chemical shifts correlated to DMSO-d_6 values.



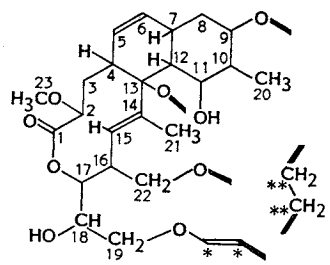
displayed in Fig. 9. A comparison of NMR chemical shifts and coupling constants for coloradocin and the corresponding values for nodusmicin (Tables 2 and 3) solidly supported the partial structure of coloradocin shown in Fig. 9.[3]

Table 2. NMR chemical shift correlations (DMSO-d_6) between coloradocin and nodusmicin.

No.	^{13}C NMR				^{1}H NMR	
	Coloradocin (ppm, multi.)		Nodusmicin (ppm, multi.)		Coloradocin (ppm)	Nodusmicin (ppm)
1	172.8	s	174.2	s	—	—
2	81.8	d	83.2	d	3.79	3.67
3	32.9	t	36.2	t	2.43 (3_a)	2.3 (3_a)
					1.22 (3_b)	1.3 (3_b)
4	37.7	d	44.1	d	2.16	2.1
5	128.3	d	133.8	d	5.51	5.51
6	129.9	d	129.9	d	5.82	5.86
7	29.2	d	39.4	d	2.14	2.46
8	27.5	t	86.1	d	1.21 (8_a)	3.84
					1.66 (8_b)	—
9	69.9	d	72.7	d	3.52	3.47
10	38.8	d	51.0	d	1.85	1.9
11	75.3	d	75.4	d	3.18	3.3
12	37.9	d	36.5	d	1.97	2.2
13	75.9	s	89.8	s		
14	141.5	s	136.8	s		
15	121.6	d	131.2	d	5.85	5.18
16	36.4	d	33.9	d	3.05	2.87
17	72.5	d	79.9	d	5.26	4.92
18	65.0	d	66.0	d	4.26	3.7
19	71.8	t	22.8	q	4.09 (CH_2)	1.08 (CH_3)
20	15.6	q	15.0	q	0.85	0.84
21	14.7	q	18.6	q	1.64	1.74
22	63.7	t	17.1	q	4.79 (22_a)	1.13
					3.97 (22_b)	—
23	57.2	q	58.5	q	3.18	3.16

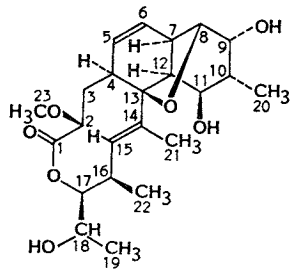
multi.: Multiplicity of signals.

Fig. 9. Comparison of partial structure of coloradocin with nodusmicin.



Coloradocin (disconnections —)

Disconnected carbon singlets
171.0, 165.5, 164.1, 137.3, 133.7



Nodusmicin

Re-examination of the IR spectrum of coloradocin became a key step toward obtaining a complete structure of this molecule. We concluded that the two bands at 1835 cm^{-1} (weak) and 1760 cm^{-1} (strong) could be due to symmetrical and antisymmetrical stretching frequencies respectively of a substituted maleic anhydride type functionality.[16] This conclusion was supported by the results of a Raman spectrum of coloradocin in the solid state. As expected,[17] the intensity of the symmetrical

Table 3. ^{1}H-^{1}H coupling correlations (DMSO-d_6) between coloradocin and nodusmicin.

Coupled protons	Coloradocin J (Hz)	Nodusmicin J (Hz)
2, 3_a	12.0	10.8
2, 3_b	4.4	4
3_a, 3_b	14.9	14.8
3_a, 4	1.7	1
3_b, 4	<1	1
4, 5	2.5	2.8
5, 6	10.0	9
6, 4	2.0	1
6, 7	6.6	7
7, 12	0	0
8_a, 7	0	0
8_b, 7	8	—
8_a, 9	0	5
8_b, 9	5.4	—
9, 10	2.0	5
10, 11	7.5	8.5
10, 20	7.5	7
11, 12	2	2
15, 15	6.6	7
15, 21	—	1
16, 17	5.8	5
17, 18	10.8	8

Table 4. Characteristic Raman frequencies (cm^{-1}) for coloradocin and citraconic anhydride.[17]

	Coloradocin	Citraconic anhydride
Symmetrical C=O	1835	1835
Antisymmetrical C=O	1760	1765
Other strong bands	1642, 658	1646, 655

Fig. 10. Partial structure of coloradocin.

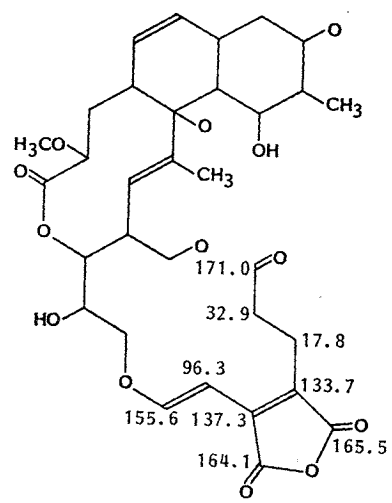


Table 5. ^{13}C and ^{1}H NMR chemical shifts (ppm) of coloradocin (DMSO-d_6).

No.	^{13}C	^{1}H
1	172.8	—
2	81.8	3.79
3	32.9	2.43 (3_a), 1.22 (3_b)
4	37.7	2.16
5	128.3	5.51
6	129.9	5.82
7	29.2	2.14
8	27.5	1.21 (8_a), 1.66 (8_b)
9	69.6	3.52
10	38.8	1.85
11	75.3	3.18
12	37.9	1.97
13	75.9	—
14	141.5	—
15	121.6	5.85
16	36.4	3.05
17	72.5	5.26
18	65.0	4.26
19	71.8	4.09 (19_a, 19_b)
20	15.6	0.85
21	14.7	1.64
22	63.7	4.79 (22_a), 3.97 (22_b)
23	57.2	3.18
24	171.0	—
25	32.9	2.43 (25_a), 1.22 (25_b)
26	17.8	2.61 (26_a), 3.37 (26_b)
27	133.7	—
28	165.5	—
29	164.1	—
30	137.3	—
31	96.3	5.62
32	155.6	7.71

Fig. 11. Structure of coloradocin.

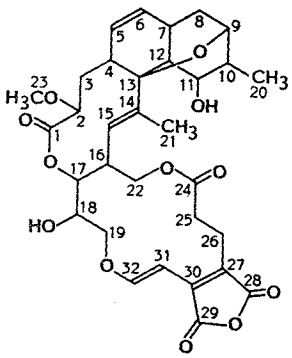
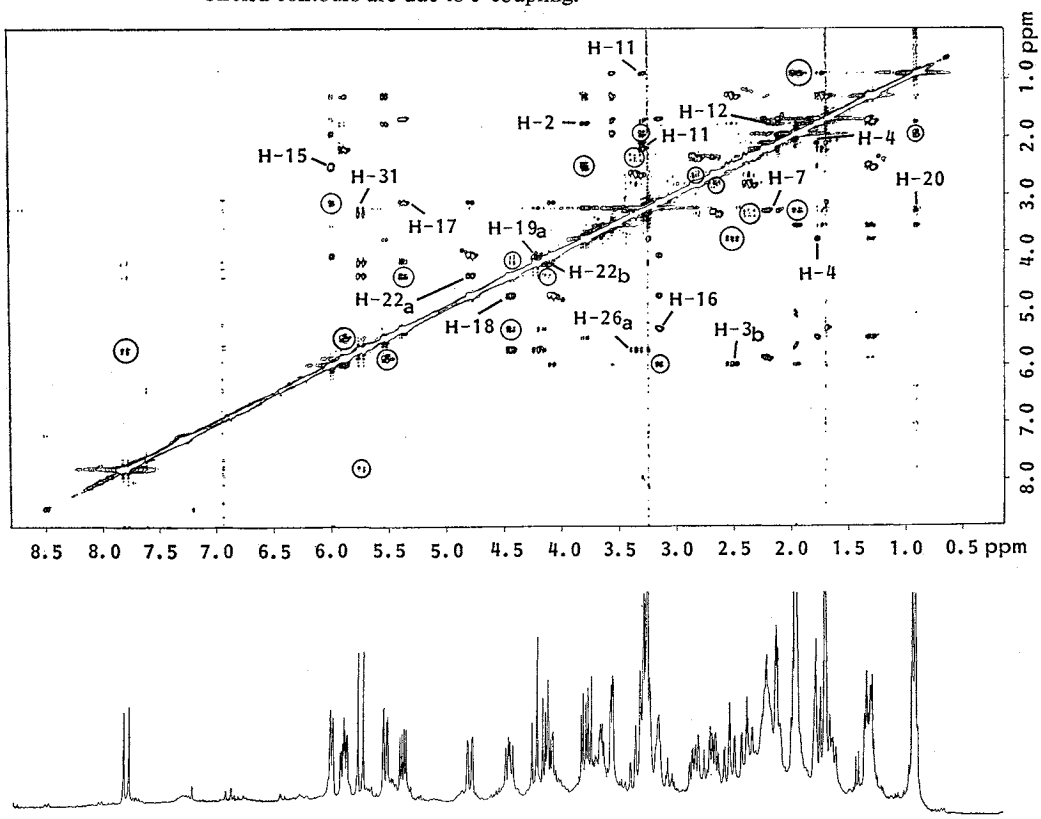


Fig. 12. 2D NOE (CAMEL) NMR spectrum (300 MHz) of coloradocin in CD_3CN. Circled contours are due to *J*-coupling.

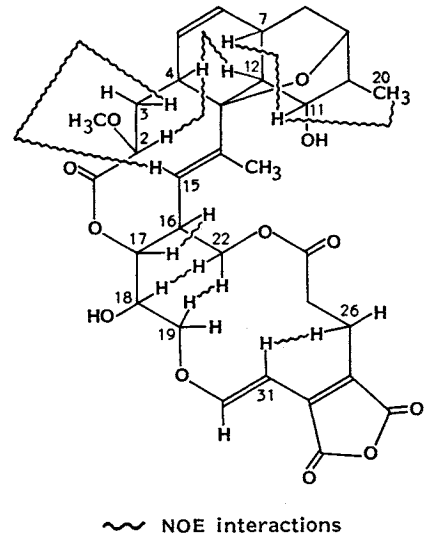


band (1835 cm^{-1}) was now greater than that of the antisymmetrical band (1760 cm^{-1}). Also, two other intense bands at lower frequency were in close agreement with two correspondingly strong bands for citraconic anhydride (Table 4). The fact that the FUCOUP experiment demonstrated long range coupling between the carbon at 164.1 ppm and the proton (5.62 ppm) on the carbon at 96.3 ppm and also between the carbon at 165.5 ppm and one of the protons (3.37 ppm) on the carbon at 17.8 ppm, allowed us to formulate a structure in which the olefinic anhydride carbons (133.7 and 137.3 ppm) were connected to the carbons at 96.3 ppm and 17.8 ppm (Fig. 10). This provided a chromophore consistent with λ_{max} 344 nm. An estimated λ_{max} 320 nm for this chromophore (not including stereochemical effects) was calculated as follows: 290 nm[18] (maleic anhydride) +13 nm[19] (value for an additional double bond in conjugation) +17 nm[20] (oxygen as part of a conjugated system). A connectivity between the carbon at 32.9 ppm which was one removed from the chromophore, and the carbon at 171.0 ppm was made on the basis of INADEQUATE NMR results for ^{13}C enriched coloradocin.† This connectivity was corroborated by a long range (2-bond) ^{13}C-^{1}H coupling (FUCOUP) between the carbon at 171.0 ppm and the proton (2.61 ppm) on the carbon at 32.9 ppm and also a 3-bond coupling between the same carbon and the proton (2.83 ppm) on the carbon at 17.8 ppm (Fig. 10). To satisfy the molecular formula of coloradocin it was necessary to form an ester linkage between the disconnected carbonyl (171.0 ppm) and one of the 3 oxidized carbons shown in Fig. 10;

† NMR results on doubly ^{13}C-labeled coloradocin and the biosynthetic implications will be discussed in a future publication.

then by default, the remaining two would be coupled as an ether. A 2D NOE (CAMEL) NMR experiment provided solid evidence for a 14-membered macrolactone resulting from an ester linkage at C-22, thereby, requiring that C-9 and C-13 become linked through oxygen. Our proposed structure for coloradocin and numbering system is shown in Fig. 11 and all ^{1}H NMR and ^{13}C NMR chemical shift assignments are shown in Table 5. The 2D NOE contour map is shown in Fig. 12 along with the accompanying ^{1}H NMR spectrum on the X-axis. Several significant cross peaks are labeled in Fig. 12, and the interactions they represent are diagrammed in Fig. 13. In addition to offering support for the position of closure to form a 14-membered macrolactone, these data also offered insight into the stereochemistry of the remainder of the molecule. For example, NOE's between H-4 and H-12, H-7 and H-11, H-11 and H-20, supplemented by the fact that the decalin bridgehead protons were in a *cis* orientation ($J_{7,12}=0$, Table 3), implied that the stereochemistry of this part of the molecule (with the exception of C-9) was the same as nodusmicin. On steric grounds, C-9 had to have an inverted stereochemistry because the ether bridge could only form across the face of the molecule opposite the bridgehead protons. The stereochemistry of the 10-membered macrolactone also appeared tobe the same as that of nodusmicin with the possible exception of C-16 and C-17. The NOE between H-2 and H-4 (already shown to be below the plain of the ring) placed the methoxy group, by inference, above the plain of the ring. The NOE between H-15 and H-3_b implied that coloradocin had a *trans* stereochemistry at C-14 and C-15 as in nodusmicin. Although there was an NOE between H-16 and H-17, multiple possible interpretations of this result prevented us from making unambiguous stereochemical assignments. Chemical derivatization and degradation studies are in progress in an attempt to verify our proposed structure for coloradocin.

Fig. 13. NOE interactions of coloradocin based on the CAMEL NMR experiment.



Acknowledgments

The authors thank Drs. J. HOCHLOWSKI and L. MITSCHER (University of Kansas) for helpful discussions; Mr. G. CLEARY and Mr. R. ANDERSON for IR and Raman analyses.

References

1) JACKSON, M.; J. P. KARWOWSKI, R. J. THERIAULT, P. B. FERNANDES, R. C. SEMON & W. L. KOHL: Coloradocin, an antibiotic from a new *Actinoplanes*. I. Taxonomy, fermentation and biological properties. J. Antibiotics 40: 1375~1382, 1987
2) ŌMURA, S.; R. IWATA, Y. IWAI, S. TAGA, Y. TANAKA & H. TOMODA: Luminamicin, a new antibiotic. Production isolation and physico-chemical and biological properties. J. Antibiotics 38: 1322~1326, 1985
3) RASMUSSEN, R. R.; M. H. SCHERR, J. B. MCALPINE, D. N. WHITTERN, A. M. BUKO, H. J. R. WEINTRAUB & E. T. OLEJNICZAK: Coloradocin: a new antibiotic from actinoplanes. II. Isolation and determination of structure. Program and Abstracts of the 26th Intersci. Conf. on Antimicrob. Agents Chemother., No. 911, p. 264, New Orleans, Sept. 28~Oct. 1, 1986

4) BAX, A.; R. FREEMAN & G. MORRIS: Correlations of proton chemical shifts by two-dimensional Fourier transform NMR. J. Magn. Reson. 42: 164~168, 1981
5) FREEMAN, R. & G. A. MORRIS: Experimental chemical shift correlation maps in nuclear magnetic resonance spectroscopy. J. Chem. Soc. Chem. Commun. 1978: 684~686, 1978
6) BODENHAUSEN, G. & R. FREEMAN: Correlation of proton and carbon-13 NMR spectra by heteronuclear two-dimensional spectroscopy. J. Magn. Reson. 28: 471~476, 1977
7) BOTHNER-BY, A. A.; R. L. STEPHENS, J. LEE, C. D. WARREN & R. W. JEANLOZ: Structure determination of a tetrasaccharide: Transient nuclear Overhauser effects in the rotating frame. J. Am. Chem. Soc. 106: 811~813, 1984
8) RICHARZ, R.; W. AMMANN & T. WIRTHLIN: Computer-assisted determination of carbon connectivity patterns in organic molecules from natural-abundance J_{cc} data. J. Magn. Reson. 45: 270~283, 1981
9) DODDRELL, D. M.; D. T. PEGG & M. R. BENDALL: Distortionless enhancement of NMR signals by polarization transfer. J. Magn. Reson. 48: 323~327, 1982
10) RASMUSSEN, R. R. & M. H. SCHERR: Preparative low-pressure chromatography of antibiotics on a column of diol-bonded silica gel. J. Chromatogr. 386: 325~332, 1987
11) ITO, Y.: High-speed countercurrent chromatography. CRC Crit. Rev. Anal. Chem. 17: 65~143, 1987
12) BRILL, G. M.; J. B. MCALPINE & J. E. HOCHLOWSKI: Use of coil planet centrifuge in the isolation of antibiotics. J. Liq. Chromatogr. 8: 2259~2280, 1985
13) ŌMURA, S.: A. Nargenicin and nodusmicin with a 10-membered lactone. Chapter 13. Production, structure, and biological properties of macrolide-like antibiotics. *In* Macrolide Antibiotics. Chemistry, Biology, and Practice. *Ed.*, S. ŌMURA, p. 512, Academic Press, New York, 1984
14) WHALEY, H. A.; C. G. CHIDESTER, S. A. MIZSAK & R. J. WNUK: Nodusmicin: The structure of a new antibiotic. Tetrahedron Lett. 21: 3659~3662, 1980
15) CELMER, W. D.; G. N. CHMURNY, C. E. MOPPETT, R. S. WARE, P. C. WATTS & E. B. WHIPPLE: Structure of natural antibiotic CP-47,444. J. Am. Chem. Soc. 102: 4203~4209, 1980
16) BELLAMY, L. J.: The Infra-red Spectra of Complex Molecules. Vol. 2. pp. 133~135, Chapman and Hall, New York, 1980
17) DOLLISH, F. R.; W. G. FATELEY & F. F. BENTLEY: Characteristic Raman Frequencies of Organic Compounds. pp. 207~208, Wiley, New York, 1974
18) WHEELER, O. H. & L. A. KAPLAN (*Ed.*): Organic Electronic Spectral Data. Vol. III. p. 13, Interscience, New York, 1966
19) HUFF, R. K.; C. E. MOPPETT & J. K. SUTHERLAND: The nonadrides. Part VI. Dimerization of the C_9 unit *in vivo* and *in vitro*. J. Chem. Soc. Perkin Trans. I 1972: 2584~2590, 1972
20) RONDESTVEDT, Jr., C. S.; M. J. KALM & O. VOGL: Arylation of unsaturated systems by free radicals. VII. The Meerwein reaction. V. Further arylations of maleimides. Ultraviolet spectra of arylmaleimides, arylmaleic anhydrides and arylmaleo- and fumaronitriles. J. Am. Chem. Soc. 78: 6115~6120, 1956