An Unusual Cyclization Product from the Reaction of Citreamicin η with Sulfene[1]

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A recent report from our laboratories described the isolation, purification, and structure determination of the citreamicin (LL-E19085) antibiotics.[2] These compounds are of interest due to their potent antibacterial activity[3] and novel structure. In the course of analog preparation, citreamicin η (**2**)[13] was reacted with methanesulfonyl chloride/triethylamine. The resultant sulfene formed both a methanesulfonate ester with the hydroxymethyl group at C(1) and a cyclic β-hydroxy sulfonate with the phenolic hydroxyl group. We describe herein the preparation and structure elucidation of this novel product.

Results and Discussion

The citreamicin antibiotic complex was prepared by fermentation, and the various components were separated as previously described.[2] Citreamicin α (**1**) comprised the major product. Although citreamicin η (**2**) is a natural product, it was present in only small amounts in the fermentation mixture. When a larger quantity was desired, it was conveniently obtained by acid catalyzed methanolysis of the α component. Reaction of **2** with $CH_3SO_2Cl/(C_2H_5)_3N$ at low temperature[4] gave a new product whose ^1H and ^{13}C NMR spectral characteristics were different from those expected for a simple bismesylate.

The NMR data for compounds **2** and **3** are given in Table I. Only a single CH_3SO_3 resonance was seen at δ 3.19, and a new pair of one-proton doublets was seen at δ 4.62 and 5.33 (J = 14.5 Hz) in the proton spectrum of **3**. In the carbon spectrum, new resonances were seen at δ 36.8 and 60.3.

The data compiled from the ^1H, ^{13}C, DEPT, HETCOR and LR-HETCOR (or HMBC) experiments were interpreted in a manner similar to that described in ref 2. LR-HETCOR (or HMBC), by showing correlations between protons and carbons two and/or three bonds apart, provided the most information on relative locations of the carbon and hydrogen atoms.

The DEPT of **3** indicated that the new resonances (60.3, 36.8 ppm) were due to CH_2 and CH_3 carbons, respectively. The HETCOR results showed the CH_2 carbon correlating with two protons resonating at 5.33 and 4.62 ppm (H(15), H(15′)). The LR-HETCOR experiment had crosspeaks at 5.33, 141.6; 5.33, 61.3; and 4.64, 69.3 ppm. The selective INEPT or SINAPT experiment gave results indicating that the 5.33 ppm resonance was coupled to the 69.3, 126.8, and 141.6 ppm carbon resonances. The latter indicated that reaction of sulfene with the phenolic OH and CH_2OH groups did not occur in the same manner. The presence of a 6.8 ppm resonance in the ^1H spectrum of **3** and its disappearance in the HETCOR spectrum implied a new OH group.

Correlation between the ^1H (8.31 ppm) and the ^{13}C (141.6 ppm) resonances aided in fixing their associated atoms into the molecular framework. The ^1H (8.31)/^{13}C (125.2) pair can be assigned to the 7-position based on previous NMR work with the precursors and three multibond ^1H–^{13}C correlations from the LR-HETCOR and SINAPT data. The structure shown in Figure 1 and the assignments set out in Table I are consistent with all of the spectral data and with the assumption that the ^1H (8.31)/^{13}C (125.2) resonances can be assigned to position 7.

An experiment was also carried out to confirm that C(15) was adjacent to C(14b). It was possible that incorrect assignment of the carbon resonances of framework atoms could lead to positional interchange of the sulfur and methylene groups in the 7-membered ring. This alternate structure would still be consistent with the LR-HETCOR and SINAPT data. However, the methylene carbon would no longer be adjacent to any other carbon atom. Compound **4** was synthesized using ^{13}C-enriched methanesulfonyl chloride and a one-dimensional INADEQUATE experiment was carried out. This experiment produces peaks (antiphase doublets) only from ^{13}C nuclei coupled to other ^{13}C nuclei, a very rare situation at natural isotope abundance levels. The enrichment increased the chance by a factor of nearly 100 that the 69.3 and 60.3 ppm resonances would be coupled (if associated carbons were adjacent). The one-dimensional INADEQUATE spectrum displayed antiphase doublets only at 60.3 and 69.3 ppm, indicating ^{13}C–^{13}C coupling and direct bonding of the two associated carbons.

The conclusions drawn from these NMR studies were subsequently confirmed by single crystal X-ray analysis of **3**.[5] The X-ray work also provided the relative configuration of the substituents at C(1) and C(3a). The molecular structure of **3** is shown in Scheme I. C(1) contained the expected primary methanesulfonate ester. In addition, a 7-membered cyclic sulfonate had formed, linking the phenol and quinone rings. Reaction of sulfene with the phenolic hydroxyl group produces an intermediate carbanion ($-OSO_2CH_2^-$). Due to the nature of the polycyclic ring system, this species must be positioned within bonding distance of the quinone carbon. A covalent bond can then form as shown in Scheme II.

A related two-step sequence in carbohydrate chemistry has been previously described.[6] Mesylation of furanose cyanohydrins (methanesulfonyl chloride/pyridine) yielded α-(mesyloxy) nitriles. Treatment with base produced a

† Infectious Diseases and Molecular Biology Research Section.
‡ Analytical Services Department.

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(5) The X-ray crystal data collection, structure solution, and refinement were carried out by Dr. Hattie H. Liu at Molecular Structure Corporation, TX. The X-ray data have been deposited with the Cambridge Crystallographic Data Centre. This material can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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Table I

2[a]			3[a]		
atom no.	carbon chem. shift (PPM)	proton chem. shift (PPM)	atom no.	carbon chem. shift (PPM)	proton chem. shift (PPM)
1	63.4		1	62.8	
C(1)–CH_3	18.4	1.67	C(1)–CH_3	18.6	1.81
1a	65.8	4.46	1a	69.9	4.78, 4.52 (d, J = 10.5 Hz)[b]
2	171.9		2	171.4	
3a	93.6		3a	94.1	
C(3a)–CH_3	25.1	1.66	C(3a)–CH_3	25.5	1.49
4	40.2	3.48, 3.35	4	41.6	3.73 (d, J = 14.4 Hz)[b]
4a	135.6		4a	136.0	
5	117.8	7.48	5	127.1	8.08
5a	140		5a	138.8	
6	132.3	8.17	6	130.7	8.25 (d, J = 8.6 Hz)[b]
7	124.2	8.17	7	125.2	8.31 (d, J = 8.6 Hz)[b]
7a	130.0		7a	128.2	
8	177.6		8	177.6	
8a	154.6		8a	149.3	
9a	150.5		9a	151.0	
10	101.2	7.44	10	100.6	7.36
11	155.3		11	156.1	
C(11)–OCH_3	56.8	3.98	C(11)–OCH_3	56.9	3.96
12	148.3		12	148.4	
C(12)–OCH_3	56.0	3.91	C(12)–OCH_3	56.1	3.89
13	104.1	7.45	13	103.8	7.45
13a	118.7[c]		13a	117.6	
14	171.9		14	175.5	
14a	119.6		14a	126.8	
15	180.7		14b	69.3	6.8 (OH)
15a	136.7		15	60.3	5.33, 4.62 (d, J = 14.5 Hz)[b]
15b	118.6[c]		17a	148.1	
16	160.9	13.5 (OH)	17b	121.3	
16a	107.1		17c	123.1	
17	165.7		17d	141.6	
			18	158.0	
			O_2CH_3	36.8	3.19
			15	60.3	5.33, 4.62 (d, J = 14.5 Hz)[b]

[a] Compound dissolved in DMSO-d_6. [b] Coupling measured in $CDCl_3$. [c] May be interchangeable. Digital resolution not sufficient in HMBC to distinguish the two.

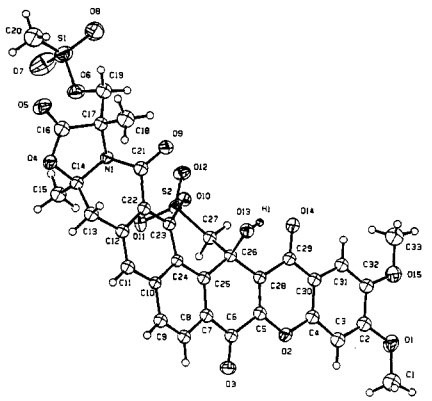


Figure 1. ORTEP plot of **3** showingthe atomic numbering scheme. The DMF solvate molecule has been omitted.

spiro 4-amino-1,2-oxathiole 2,2-dioxide via formation of an $-OSO_2CH_2^-$ species followed by nucleophilic attack on the nitrile.

Experimental Section

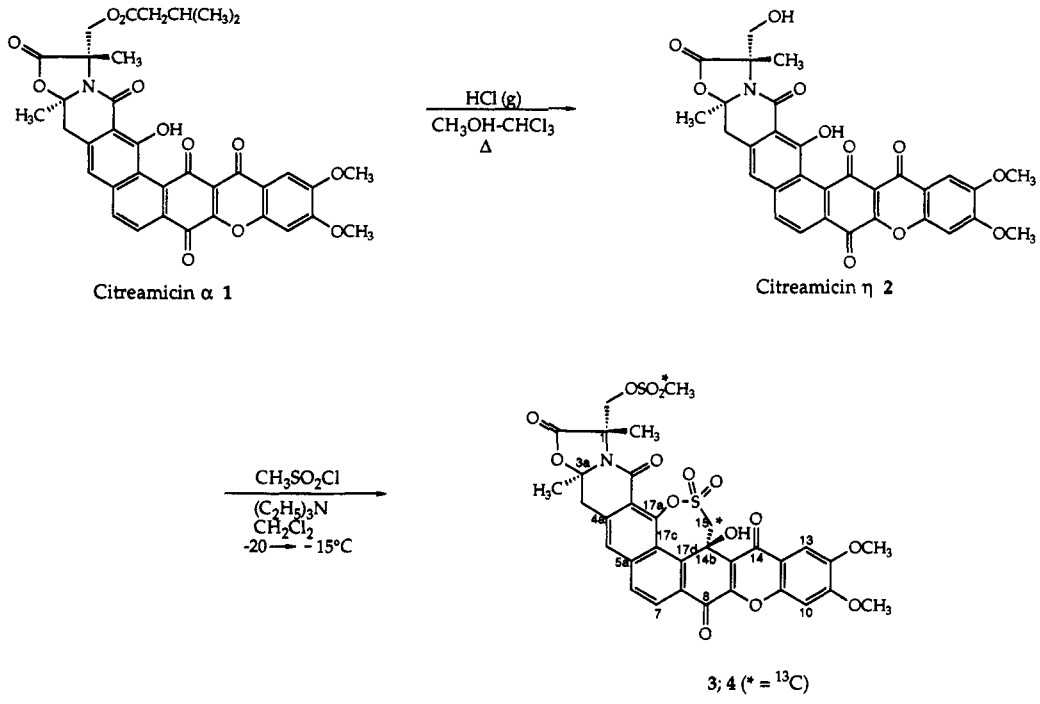
General. Unless otherwise noted, materials were obtained from commercial sources and were used without further purification. The $^{13}CH_3SO_2Cl$ (99.5 atom %) was custom synthesized by MSD Isotopes. Column chromatography was done on silicic acid (Bio-Sil A, 200–400 mesh, Bio-Rad Laboratories). Thin-layer chromatography was done on silica gel plates (Whatman silica gel 60A) containing fluorescent indicator. The solvents were $CHCl_3$–MeOH mixtures. Organic extracts were dried over anhyd Na_2SO_4. NMR spectra were done at 500 and 300 MHz. High resolution mass spectra (HRFABMS) were obtained in the fast atom bombardment mode. Samples dissolved in MeOH were added to a matrix of dithiothreitol/dithioerythritol (5/1 w/w) on the probe tip.

***cis*-3a,4-Dihydro-16-hydroxy-1-(hydroxymethyl)-11,12-dimethoxy-1,3a-dimethyl[1]benzopyrano[2′,3′:6,7]napth[2,1-g]oxazolo[3,2-b]isoquinoline-2(1H),8,14,15,17-pentone (Citreamicin η, 2).** Citreamicin α (1; 1.20 g, 1.79 mmol) dissolved in a small volume of $CHCl_3$ was diluted with $CHCl_3$–MeOH (1:4, 1200 mL) using rapid overhead stirring to produce a red flocculent precipitate. An additional 36 mL of $CHCl_3$–MeOH saturated with HCl gas was added to the reaction mixture and refluxed for 24 h. The methanolysis mixture was then chilled, and the precipitated product was collected by filtration, washed with MeOH, and dried *in vacuo*. This crude material was dissolved in CH_2Cl_2–MeOH, preabsorbed onto Bio-Sil A (12 g) and thoroughly dried. The mixture was placed on top of a Bio-Sil A flash chromatography column and eluted with CH_2Cl_2, 1% MeOH in CH_2Cl_2 (elutes some starting ester) and 2% MeOH in CH_2Cl_2 (elutes product). There was obtained 0.850 g (81%) of red-orange solid:

$[\alpha]^{26}_D$ −108° (*c* 1.11, DMSO); IR (KBr) 3527, 1799, 1693, 1620, 1508, 1458, 1427, 1377, 1275, 1234, 1047 cm^{-1}; UV $\lambda_{max}^{CH_3OH}$nm (ϵ) 222 (5.74×10^4), 250 (4.36×10^4), 321 (3.66×10^4), 383 (1.15×10^4); HRFABMS for $C_{31}H_{24}NO_{11}$ ($M^+ + H$) requires *m/e* 586.1349, found 586.1345.

(1α,3aα,14bβ)-3a,4,14b,15-Tetrahydro-14b-hydroxy-11,12-dimethoxy-1,3a-dimethyl-1-[[(methylsulfonyl)oxy]methyl]-18*H*-[1]benzopyrano[3′,2′:5,6]naphth[1,8-*de*][1,2]-(−)-oxathiepino[10,1-*gh*]oxazolo[3,2-*b*]isoquinoline-2(1*H*),8,14,18-tetrone 16,16-Dioxide (3). Citreamicin η (2; 0.250 g, 0.427 mmol) was slurried in 20 mL of dry CH_2Cl_2 under argon and chilled to −19 °C. Triethylamine (0.297 mL, 0.216 g, 2.14 mmol) was added, followed by methanesulfonyl chloride (0.166 mL, 0.246 g, 2.14 mmol; dropwise). A TLC at 0.5 h showed no **2**. After 1 h (T =

Scheme I

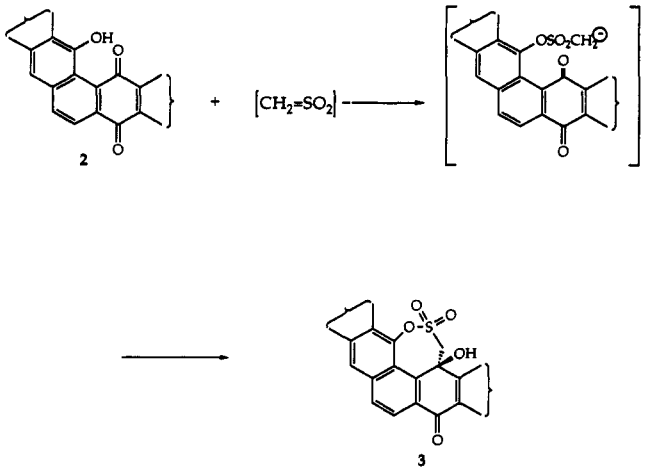


Citreamicin α 1

Citreamicin η 2

3; 4 (* = ^{13}C)

Scheme II



2

3

-14 °C), the reaction was poured into ice–water. The organic layer was removed and washed with cold 10% HCl and 10% $NaHCO_3$, dried, and evaporated. The crude product was dissolved in 0.75% MeOH in CH_2Cl_2 and purified by flash chromatography on Bio-Sil A. There was obtained 0.210 g (66%) of yellow solid: $[\alpha]^{26}_D$ +197° (*c* 1.02, DMSO); IR (KBr) 3430, 1807, 1681, 1645, 1624, 1509, 1478, 1457, 1434, 1384, 1362, 1324, 1276, 1175 cm^{-1}; UV $\lambda_{max}^{CH_3CN}$nm (ε) 238 (7.04 × 10^4), 292 (3.06 × 10^4), 397 (1.12 × 10^4); HRFABMS for $C_{33}H_{28}NO_{15}S_2$ (M^+ + H) requires *m/e* 742.0900, found 742.0910.

The ^{13}C labeled material (4) was prepared in an identical manner starting with $^{13}CH_3SO_2Cl$. HRFABMS for $C_{33}H_{28}NO_{16}S_2$ (M^+ + H containing 2 ^{13}C atoms) requires *m/e* 744.0967, found 744.0985.

NMR Methods. The NMR samples were prepared by adding solid 2 or 3 to 0.6 mL of DMSO-d_6 at 80 °C to saturate the solution. Upon cooling to 25 °C, no precipitate formed, and the solution was transferred to an NMR sample tube. One-dimensional ^1H, ^{13}C and DEPT[7] experiments were then performed. All chemical shifts were obtained from the high resolution one-dimensional spectra. TMS was used as the primary chemical shift reference in the ^1H spectra. The DMSO-d_6 ^{13}C peak at 39.5 ppm was used as a secondary reference for chemical shifts in the ^{13}C spectra.

HMBC experiments were performed as described in ref 8, and DEPT and HETCOR techniques are given in ref 9. Details of the NMR pulse sequences are included in the supplementary material. The one-dimensional INADEQUATE experiment was done according to ref 10, the LR-HETCOR procedure followed ref 11, and the INEPT (SINAPT) experiment was done as in ref 12.

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Supplementary Material Available: ORTEP plot of 3, NMR and X-ray crystallographic methods, and ^1H NMR spectra (500 MHz) for compounds 2-7 (7 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(13) The absolute stereochemistry of these molecules is unknown. Only a single enantiomer is shown in the schemes.