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Access to lincomycin *N*-oxide isomers controlled by reaction conditions

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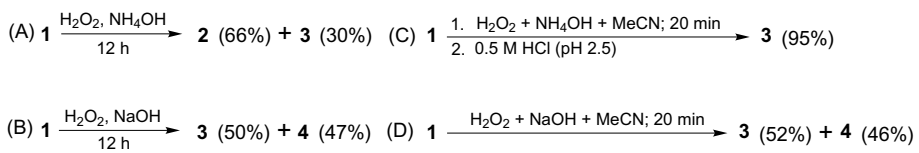
Abstract—Oxidation of lincomycin with H_2O_2 in alkaline media leads to *N*-oxides, besides the conversion of thiomethyl group into sulfoxides and sulfones. NH_4OH favors formation of the *S*-isomer; both *R*- and *S*-isomers of the *N*-oxide are formed in the presence of NaOH. Addition of acetonitrile markedly accelerates the reaction.

Lincomycin **1**, an aminoglycoside antibiotic from *Streptomyces lincolnensis*,[1] and its semisynthetic 7-chloro-7-deoxy analogue (clindamycin[2]) are widely used in human and veterinary medicine.[3] Many attempts to modify these compounds have been reported.[4] Sulfoxides of lincomycin[5] and clindamycin[6] were initially obtained by microbial transformation. Two isomeric sulfoxides were prepared in low yield by oxidation of **1** with dimethyloxirane. Their further oxidation with OsO_4 and *N*-methylmorpholine *N*-oxide gave the corresponding sulfones.[7] There are two possible sites for oxidative attack in lincomycin: the thiomethyl group and the pyrrolidine nitrogen. Tertiary amines are known to be oxidized slowly by hydrogen peroxide.[8,9] However, Sztaricskai et al.[7] reported that peracids, $NaIO_4$ and H_2O_2 are unsuitable for the oxidation of **1**. Nevertheless, in our recent work we described the preparation of the sulfoxide and sulfone derivatives of **1**[10] using H_2O_2 under mild acid or neutral conditions for 24 h.

We looked for reaction conditions, which would also lead to the oxidation of the tertiary amine in **1**. New substances, slowly formed with aqueous H_2O_2 under slightly basic conditions, were accompanied by a considerable amount of degradation products.[11] Raising the pH above 9.5 resulted in a crucial improvement. The outcome depended on the base used: with NH_4OH (method A),[12] compounds **2** and **3** were formed, **3** and **4** were produced when NaOH (method B)[13] was used (Scheme 1). These compounds were isolated from reactions performed on a preparative scale and their structures were elucidated by MS and NMR analyses.

Compounds **2**, **3** and **4** exhibited intense $[M+H]^+$ peaks in positive-ion mode MALDI MS at *m*/*z* 439, 455 and 455, respectively (Scheme 2). During a post-source decay (PSD) experiment, allowing analysis of fragment ions, compound **2** lost a CH_3SO moiety (63 Da) and formed the ion *a* (*m*/*z* 376). The same fragment ion arose by the elimination of a CH_3SO_2 unit (79 Da) from the molecular ions of **3** and **4**. The diagnostic ion *b* (*m*/*z* 170), occurring in PSD spectra of all three compounds, was formed by the cleavage of the amide bond and confirmed the introduction of one oxygen in the pyrrolidine



Scheme 1. Preparation of lincomycin *N*-oxides.

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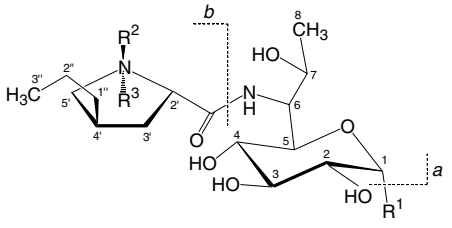

No.	R^1	R^2	R^3	$[M+H]^+$	*a* ion	*b* ion
1	CH_3S	CH_3	—	407.2	360.2	—
2	CH_3SO	CH_3	O	439.2	376.2	170.1
3	CH_3SO_2	CH_3	O	455.2	376.2	170.1
4	CH_3SO_2	O	CH_3	455.2	376.2	170.1

Scheme 2. Structure, atom numbering and mass spectrometric fragmentation of lincomycin **1** and its oxidation products.

moiety; no such fragment was observed in the spectrum of the parent compound. The abundant ion m/z 126 including the nitrogen-containing ring was common to all the compounds. Therefore, **2** contains both sulfoxide and *N*-oxide groups; **3** and **4** are isomeric *N*-oxide sulfones. The location of the introduced oxygen atoms and the nature of the newly formed groups could also be inferred from a combination of the ^1H and ^{13}C NMR data (Table 1). The nature of the methyl singlets was obtained from their heteronuclear coupling pattern observed in HMBC: the *S*-methyl was coupled to C-1 only, but the *N*-methyl showed cross peaks to C-2′ and C-5′. Furthermore, the *S*-methyl and H-1 had a cross peak in the ROESY spectrum. Considering the chemical shift of the corresponding carbons, all compounds are *N*-oxides; **2** is a sulfoxide and both **3** and **4** contain a CH_3SO_2 group. This deduction is supported by the large downfield shifts of C-2′ and C-5′ with respect to the parent compound. The comparison of the NMR data showed a great similarity in the *galacto*-octopyranose part. However, the conformations of pyrrolidinium ring are different in the isomeric pair **3** and **4**, judging by the vicinal coupling constants; that of **2** is similar to **3**. Whereas the chemical shift differences are small for the *N*-methyl and C-5′ (0.9 and 0.8 ppm), those of C-2′ and C-4′ are large (6.3 and 1.6 ppm). The magnetic nonequivalence of the diastereotopic H-3′ and H-5′ protons was also different (0.315 and 0.336 ppm in **3**, 0.887 and 0.009 ppm in **4**; in **1** 0.05 and 0.95 ppm). An NOE between *N*-Me and H-1″ was found in **2** and **3** indicating that this group and the *n*-propyl side chain are located on the same side of the pyrrolidinium ring. Thus, **2** and **3** have an *S* configuration at N-1. Similarly, according to a cross peak between the *N*-methyl and H-4′ observed in **4**, these atoms are on the same side of the ring, which implies an *R* configuration for this compound.

The presence of acetonitrile increases the rate of oxidation of tertiary amines by aqueous hydrogen peroxide.[14] The use of H_2O_2, ammonia and acetonitrile (method C)[15] resulted in a fast exothermic reaction producing an oxidation mixture that converted **1** into **3** and its intermediate within 20 min. This intermediate was fully transformed into **3** by careful acidification of the reaction mixture with 0.5 M HCl to pH 2.5. According to densitometry, the total yield of **3** reached nearly 95%. The presence of ammonia is indispensable for this efficient conversion, as with NaOH (method D),[15] both isomers **3** and **4** were formed and no intermediate was detectable. Acetonitrile has a beneficial effect on reaction at both the N and S centres in **1**. It promotes oxidation of tertiary amines,[14] sulfides to sulfoxides and sulfoxides to sulfones (Payne oxidation).[16] A peroxyimidic acid is believed to be the active

Table 1. ^1H and ^{13}C NMR data (400 and 100 MHz, D_2O, 30 °C)

Atom	2		3		4	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C[a]	δ_H, mult. (*J* in Hz)	δ_C[a]	δ_H, mult. (*J* in Hz)
1	95.13	4.593, d (5.7)	91.3	5.009, d (6.6)	91.3	5.004, d (6.6)
2	68.48	4.233, dd (9.9, 5.7)	66.8	4.198, dd (10.4, 6.6)	66.8	4.189, dd (10.3, 6.6)
3	70.45	3.891, dd (9.9, 3.3)	68.6	4.069, dd (10.4, 3.2)	68.7	4.046, dd (10.3, 3.2)
4	68.48	3.852, dd (3.3, 1.2)	68.2	3.810, dd (3.2, 1.1)	68.1	3.784, dd (3.2, 1.1)
5	74.87	3.672, dd (8.2, 1.2)	75.2	3.973, dd (8.9, 1.1)	74.8	3.981, dd (8.9, 1.1)
6	54.09	4.097, dd (8.2, 5.3)	53.6	4.151, dd (8.9, 4.5)	53.9	4.143, dd (8.9, 4.6)
7	66.41	3.934, dq (5.3, 6.5)	66.3	4.041, dq (4.5, 6.5)	66.5	4.004, dq (4.6, 6.9)
8	17.60	1.012, d (6.5)[b]	16.7	1.042, d (6.5)[b]	16.8	1.038, d (6.9)
Me–S	35.24	2.613, s[b]	42.5	2.973, s[b]	42.6	2.981, s[b]
C=O	169.64	—	169.5	—	169.6	—
1′-Me	54.99	3.157, s[b]	54.8	3.143, s[b]	52.9	3.056, s[b]
2′	75.66	3.949, dd (11.1, 8.4)	75.5	3.956, dd (10.8, 8.4)	81.8	3.982, dd (8.8, 4.6)
3′	33.69	2.251, ddd (13.2, 11.1, 10.3)	33.4	2.247, ddd (13.3, 10.8, 10.4)	33.4	2.489, ddd (13.4, 9.4, 8.8)
		1.934, ddd (13.2, 8.4, 4.3)		1.932, ddd (13.3, 8.4, 4.4)		1.602, ddd (13.4, 6.8, 4.6)
4′	33.95	2.493, m	33.7	2.495, m	35.3	2.581, m
5′	76.40	3.483, dd (11.6, 7.9)	76.1	3.484, dd (11.6, 7.9)	73.9	3.365, dd (19.3, 6.2)
		3.147, dd (11.6, 9.1)		3.148, dd (11.6, 10.0)		3.356, dd (19.3, 9.8)
1″	35.76	1.085, m[c]	35.5	1.251, m[c]	34.7	1.285, m[c]
2″	20.54	1.261, m[c]	20.3	1.102, m[c]	20.7	1.105, m[c]
3″	13.39	0.681, t (7.3)[b]	13.3	0.677, t (7.3)[b]	13.3	0.681, t (7.3)[b]

[a] HMQC and HMBC read outs.
[b] 3 H.
[c] 2 H.

intermediate.[17] Moreover, according to our results, the participating base substantially influences the reaction mechanism.

Oxidation of **1** with H_2O_2 in the presence of NH_4OH afforded only one isomer of the sulfoxide. The other was probably rapidly oxidized into the sulfone derivative of **1**. The method used for the determination of the absolute configuration of the sulfoxides (prepared with dimethyloxirane) was in principle based on comparison of the CD spectra of both isomers;[7] we had only one at our disposal. In addition, MS and NMR spectra were used as supporting arguments. Moreover, the reported NMR spectra[7] were measured in chloroform, a solvent in which our compounds were not soluble. Therefore, we prefer to leave this configuration question open.

In our experience, the H_2O_2 must be of high quality; otherwise impurities present totally inhibit the oxidation of **1** under any conditions. The failure of Sztaricskai et al.[7] to observe oxidation of **1** with H_2O_2 might be explainable by this observation. Our results shed new light on the preparation of chemically and pharmacologically interesting[18] *N*-oxides and makes it possible to prepare a new type of these compounds easily using nontoxic oxidants.

Acknowledgements

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References and notes

1. Mason, D. J.; Dietz, A.; DeBoer, C. *Antimicrob. Agents Chemother.* **1963**, *1962*, 554–559.
2. Magerlein, B. J.; Birkenmeyer, R. D.; Kagan, F. *Antimicrob. Agents Chemother.* **1967**, *1966*, 727–736.
3. (a) *European Pharmacopoeia*, 4th ed.; Council of Europe: Strasbourg, 2001; p 1473; (b) *British Pharmacopoeia (Veterinary)*; Her Majesty's Stationery Office: London, 1998; p 100; (c) *The United States Pharmacopoeia*, 26th revision; USP Convention: Rockville, 2002; p 1081; (d) Carter, E. L. *Semin. Cutan. Med. Surg.* **2003**, *22*, 196–211.
4. Magerlein, B. J. In *Structure–Activity Relationships Among the Semisynthetic Antibiotics. Modification of Lincomycin*; Perlman, D., Ed.; Academic: New York, 1977; pp 600–651.
5. Argoudelis, A. D.; Mason, D. J. *J. Antibiot.* **1969**, *22*, 289–291.
6. Argoudelis, A. D.; Coats, J. H.; Mason, D. J.; Sebek, O. K. *J. Antibiot.* **1969**, *22*, 309–314.
7. Sztaricskai, F.; Dinya, Z.; Batta, G.; Mocsári, A.; Hollósi, M.; Majer, Z.; Masuma, R.; Omura, S. *J. Antibiot.* **1997**, *50*, 866–873.
8. Cope, A. C.; Ciganek, E. *Org. Synth. Coll. IV* **1963**, 612–615.
9. Bergstad, K.; Bäckvall, J. E. *J. Org. Chem.* **1998**, *63*, 6650–6655.
10. Pospíšil, S.; Sedmera, P.; Halada, P.; Spížek, J. *Folia Microbiol.* **2001**, *46*, 376–378.
11. Reactions were monitored by TLC on silica gel plates (Kieselgel 60 F_{254}, Merck), mobile phase $CHCl_3$–MeOH–NH_4OH (12:3:0.1); products were detected by spraying with orcinol reagent[10] and heating to 150 °C; densitometric evaluation at 600 nm.
12. *Method A*. Aqueous H_2O_2 (Perhydrol® Merck, 30%, 4 mL, 33 mM) was added to a solution of **1**-hydrochloride (Sigma, 200 mg, 0.45 mM) in NH_4OH (25%, 6 mL, 42 mM); the resulting solution having pH 10.8. The mixture was shaken for 12 h at room temperature until **1** had completely disappeared then lyophilized and subjected to column chromatography on Toyopearl HW40F (Tosoh Corp., 3×47 cm) in water, yielding pure **2** (85 mg) and **3** (36 mg).
13. *Method B*. Aqueous H_2O_2 (Perhydrol® Merck 30%, 1.3 mL, 10.7 mM) was added to an aqueous solution (0.4 mL) of **1**-hydrochloride (Sigma, 100 mg, 0.22 mM) and the pH was adjusted with 2 M NaOH to 9.75. The mixture was shaken for 12 h at room temperature until the complete disappearance of **1**. Upon neutralization to pH 6 with 2 M HCl, lyophilization and column chromatography on silica gel 60 M (0.040–0.063 mm, Merck, 1.7×60 cm) with a mobile phase $CHCl_3$–MeOH–NH_4OH (12:3:0.1) and Sephadex LH-20 (0.025–0.1 mm, Pharmacia) column chromatography (1.7×40 cm) in water, pure **3** (42 mg) and **4** (36 mg) were obtained.
14. Laus, G. *J. Chem. Soc., Perkin Trans. 2* **2001**, 864–868.
15. *Method C*. Aqueous H_2O_2 (Perhydrol® Merck, 30%, 80 μL, 0.7 mM) was added to a solution of **1**-hydrochloride (Sigma, 9.6 mg, 21.6 μM) in water (0.1 mL), NH_4OH (0.1 mL, 25%, 0.7 mM) and CH_3CN (0.3 mL, 5.7 μM). *Method D*. As method C, NH_4OH was replaced by 2 M NaOH to yield a solution of pH 9.5.
16. Payne, G. B.; Deming, P. H.; Williams, P. H. *J. Org. Chem.* **1961**, *26*, 659–663.
17. Bethell, D.; Graham, A. E.; Heer, J. P.; Markopoulou, O.; Page, P. C. B.; Park, B. K. *J. Chem. Soc., Perkin Trans. 2* **1993**, 2161–2162.
18. Denny, W. A. *Eur. J. Med. Chem.* **2001**, *36*, 577–595.