SOLID STATE THERMAL DEGRADATION PRODUCTS OF ROSARAMICIN

Mohindar S. Puar, Doris Schumacher, Tom Mollitor,
Bernard Rosenkrantz and Robert S. Jaret

Schering-Plough Research Institute,
Bloomfield, New Jersey 07003, U.S.A.

Andrew T. McPhail

Paul M. Gross Chemical Laboratory, Duke University,
Durham, North Carolina 27706, U.S.A.

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The structures of two solid-state rearrangement products, A and B, of rosaramicin have been assigned on the basis of ^{13}C NMR spectral data and a single-crystal X-ray analysis of the acetone solvate of product B.
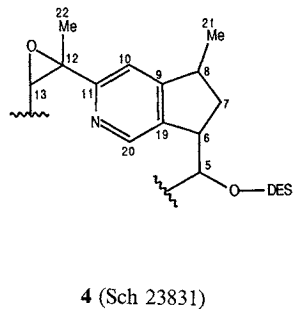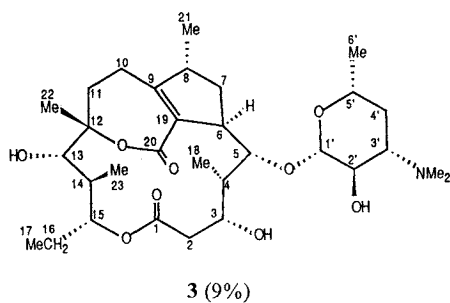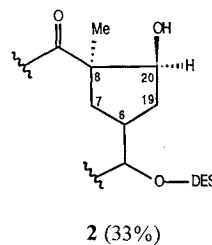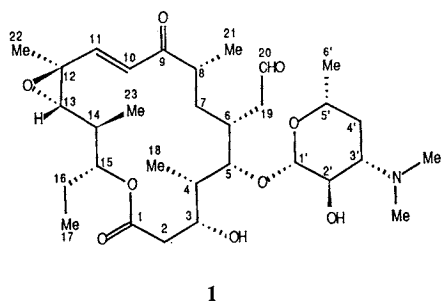
Rosaramicin 1, a sixteen-membered ring macrolide antibiotic produced by *Micromonospora rosaria*, is highly active against Gram-positive as well as Gram-negative bacteria.[1~5] When the formulated tablets[†] of 1 were subjected to heating at 75°C for extended periods of time (2~4 weeks), high pressure chromatographic analysis of the resulting material indicated that approximately 20% degradation had occurred. Tablets treated in this manner were ground and extracted with methanol and chloroform. Concentration of the combined extracts followed by chromatography of the resulting residue on silica gel furnished two major products, A (33%) and B (9%), the structures of which are reported here.

The major degradation product A, obtained in excellent purity, had the same MW (m/z 581) as 1. Its ^{1}H NMR spectrum [(CD$_3$)$_2$CO] showed the presence of methyl proton resonances at δ 0.88 (t, $J=7.0$ Hz), 1.07 (s), 1.16 (d, $J=6.5$ Hz, $2 \times$ CH$_3$), 1.21 (d, $J=6.5$ Hz), 1.50 (s) and 2.31 (s, N(CH$_3$)$_2$) and methine proton resonances at δ 2.85 (dd, $J=10$ Hz, 13-H), 3.17 (dd, $J=10$, 7 Hz, 2'-H), 3.90 (m, 3-H), 4.31 (d, $J=7$ Hz, 1'-H), 4.91 (m, $J=8.5$, 8.5, 4 Hz, 15-H), 6.29 (d, $J=16$ Hz, 10-H), 6.98 (d, $J=16$ Hz, 11-H) and 4.62 (t, $J=6.0$ Hz, 20-H). Comparison with corresponding data for 1 indicated the formation of a CH$_3$ singlet (CH$_3$CH → CH$_3$C) and a CHOH group (CHO → CHOH). Acetylation resulted in the production of a triacetate (m/z 707) in which in which protons 2'-H, 3-H and 20-H were shifted downfield to δ 4.80, 5.09 and 5.56, respectively. Structure 2 was assigned to product A.[††] ^{13}C NMR spectral data for 2 and its 3,20,2'-triacetate derivative 2a are listed in Table 1.

Product B also had a composition (m/z 581) identical with that of 1. Its ^{1}H NHR spectrum showed the presence of methyl proton resonances at δ 0.84 (t, $J=7.0$ Hz), 0.94 (d, $J=6.5$ Hz), 1.00 (d, $J=6$ Hz), 1.01 (d, $J=6.5$ Hz), 1.18 (d, $J=6.5$ Hz), 1.34 (s) and 2.25 (s, N(CH$_3$)$_2$), and methine proton resonances at δ 2.82 (d, $J=11$ Hz, 13-H), 3.12 (dd, $J=10$, 7 Hz, 2'-H), 4.31 (d, $J=7$ Hz, 1'-H), 4.00 (m, 5'-H) and

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[†]   The core formulation ingredients follow; 1, microcrystalline cellulose, sodium lauryl sulfate, corn starch, sodium starch glycolate, silicon dioxide, and magnesium stearate.

[††]   Similar products from 1 were also observed under reductive conditions (unpublished data).

1

2 (33%)

3 (9%)

4 (Sch 23831)

Table 1. ^{13}C NMR spectral data for compounds $1 \sim 4^a$.

Carbon	1	2^b	3	4^c
1	173.5	174.4 (170.0)	174.1	175.5
2	39.7	40.0 (39.2)	41.5	41.1
3	66.8	67.5 (69.2)	73.6	67.4
4	41.2	43.1 (42.2)	42.2	42.1
5	81.3	80.9 (79.9)	79.2	80.3
6	31.4	36.5 (35.7)	36.7	35.4
7	31.8	34.7 (34.0)	36.5	38.1
8	45.1	58.1 (57.6)	46.2	49.6
9	200.3	202.9 (200.5)	166.2	160.0
10	122.8	126.5 (124.7)	25.0	111.8
11	150.9	147.6 (149.9)	42.1	161.9
12 (C*)	59.7	59.8 (59.7)	85.6	62.5
13	68.0	67.7 (67.3)	86.7	72.1
14	37.8	37.9 (38.1)	40.1	36.8
15	76.8	77.7 (77.7)	76.6	79.2
16	24.7	25.0 (24.6)	23.2	25.9
17	9.0	8.8 (8.4)	9.7	8.5
18	9.0	9.9 (11.4)	11.1	9.4
19	43.9	37.0 (35.0)	132.6	138.4
20	202.9	73.6 (77.4)	171.8	144.6
21	17.4	17.5 (17.1)	17.7	18.3
22	15.0	15.2 (15.2)	5.3	16.1
23	14.5	14.6 (14.5)	24.3	14.8

^a   Carbons of the desosamine sugar are not listed.
^b   Values in parentheses are for the 3,20,2'-triacetate derivative of **2**.
^c   Sch 23831.

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[a]   Carbons of the desosamine sugar are not listed.
[b]   Values in parentheses are for the 3,20,2'-triacetate derivative of **2**.
[c]   Sch 23831.

4.70 (m, $J = 10$, 7 and 3.5 Hz, 15-H). The most important observation was the absence in this spectrum of protons due to an α,β-unsaturated moiety.

^{13}C NMR spectral data for the aglycone portion of product B indicated the presence of five methyl carbons at δ (ppm, CDCl$_3$) 5.3, 9.7, 11.1, 17.7 and 24.3, five methylene carbons at δ 23.2, 25.0, 36.5, 41.5 and 42.1, eight methine carbons at δ 36.7, 40.1, 42.2, 46.2, 73.6, 76.7, 79.2 and 86.7, while the remaining five carbons appeared as singlets at δ 85.6, 132.6, 166.2, 171.8 and 174.1. The sugar carbon resonances occurred at δ 104.5 (1'), 69.9 (2'), 65.5 (3'), 28.1 (4'), 69.9 (5'), 21.1 (6') and 40.2 (7', 8'). Comparison of the ^{13}C NMR data (Table 1) with those for **1** suggested the presence of a five-membered ring formed *via* internal cyclization (a similar ring system was also observed in **4** (Sch 23831 which was isolated from the fermentation[6]), saturation of the C$_{10}$–C$_{11}$ double bond (perhaps reduced by the CHO function which itself was oxidized to COOH group), and opening of the epoxide ring and subsequent cyclization to form a new 7- or 8-membered lactone ring involving either C$_{12}$ or C$_{13}$. A single-crystal X-ray analysis of the acetone solvate, obtained by crystallization of product B from acetone, resolved this ambiguity and provided complete details of the stereochemistry of this product. (Structure **3**)

Crystal Data for the Acetone Solvate of Product B

$C_{31}H_{51}NO_9 \cdot \frac{1}{2}C_3H_6O$ (**3**)·acetone, MW = 610.80, orthorhombic, $a = 19.577(3)$ Å, $b = 29.295(5)$ Å, $c = 5.957(1)$ Å, $V = 3489.9$ Å^3, $Z = 4$, $D_{calcd} = 1.162$ g cm^{-3}, μ(Cu-Kα radiation, $\lambda = 1.5418$ Å) = 6.6 cm^{-1}. Space group $P2_12_12(D_2^3)$ uniquely from the systematic absences: $h \neq 2n$, $0k0$ when $k \neq 2n$. Sample dimensions: $0.12 \times 0.18 \times 0.40$ mm.

Crystallographic Measurements

Preliminary unit-cell parameters and space group information were obtained from oscillation and Weissenberg photographs. One octant of intensity data was recorded on an Enraf-Nonius CAD-4 diffractometer (Cu-Kα radiation, incident-beam graphite monochromator; ω-2θ scans, $\theta_{max} = 67°$). From a total of 3,592 independent measurements, those 2,093 reflections with $I > 3.0\sigma(I)$ were retained for the structure analysis and the usual Lorentz and polarization corrections were applied. Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 25 reflections ($27° < \theta < 37°$) widely separated in reciprocal space.

Fig. 1.   Structure and solid-state conformation of thermal degradation product B; hydrogen atoms have been omitted for clarity and the broken line denotes an intramolecular O–H···O hydrogen bond.
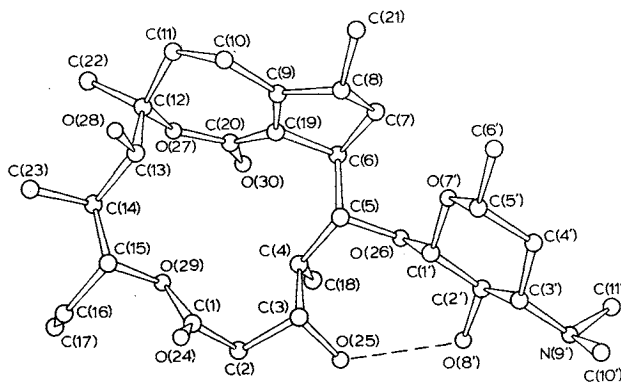
Structure Analysis

The crystal structure was solved by direct methods.[†] Approximately non-hydrogen atom positions were obtained from an E-map. Hydrogen atoms were located in a difference Fourier synthesis evaluated following several rounds of full-matrix least-squares adjustment of non-hydrogen atom positional and anisotropic thermal parameters. Continuation of the least-squares refinement, with hydrogen atoms included at their calculated positions, decreased R[††] to 0.08 at which point a difference Fourier synthesis revealed four peaks of $ca.$ $0.8\,e\,\text{Å}^{-3}$ centered on the crystallographic two-fold axis at $x=0$, $y=0$, and separated by $ca.$ $1.3\sim1.6\,\text{Å}$, and these were flanked by several smaller peaks of $ca.$ $0.3\sim0.4\,e\,\text{Å}^{-3}$. These peaks were ascribed to disordered acetone of crystallization with its $C=O$ or a $C-C$ bond vector lying approximately along the two-fold axis. Atomic positional and thermal parameters for those acetone atoms which lay on the two-fold axis were included as variables in the subsequent least-squares iterations which converged at $R=0.059$ $(R_w=0.084)$.[††] Neutral atom scattering factors used in the structure-factor calculations were taken from ref 7. In the least-squares iterations, $\Sigma w \Delta^2 (w=1/\sigma^2(|F_O|)$, $\Delta=(|F_O|-|F_C|))$ was minimized.

A view of the structure, with the atom numbering scheme, is provided in Fig. 1. In crystals of the acetone solvate, the hydroxy group at C_3 is involved in an intramolecular $O-H\cdots O$ hydrogen bond with $O_{8'}$ $(O_{25}\cdots O_{8'}=2.871(4)\,\text{Å})$ which is further linked via an intermolecular $O-H\cdots N$ hydrogen bond $(O_{8'}\cdots N_{9'}=2.862(7)\,\text{Å})$ to a molecule related by the crystallographic two-fold axis thereby producing a cavity which accommodates the acetone molecule at normal van der Waals distances. The remaining hydroxy group at C_{13} is hydrogen bonded to a molecule of 3 related by unit-translation along the c-direction $(O_{28}\cdots O_{27}=2.926(7)\,\text{Å})$.

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[†] Crystallographic calculations were performed on PDP11/44 and MicroVAX II computers by use of the Enraf-Nonius Structure Determination Package incorporating the direct methods program MULTAN11/82.

[††] $R=\Sigma||F_O|-|F_C||/\Sigma|F_O|$; $R_w=[\Sigma w(|F_O|-|F_C|)^2/\Sigma w|F_O|^2]^{1/2}$