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STRUCTURAL ELUCIDATION OF EPALRESTAT(ONO-2235), A POTENT ALDOSE REDUCTASE INHIBITOR, AND ISOMERIZATION OF ITS DOUBLE BONDS

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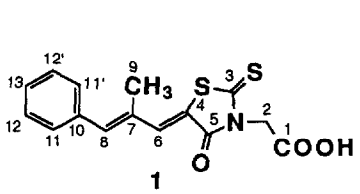
Abstract: *The structure of epalrestat (ONO-2235) is revised by X-ray single crystal analysis. The structures of the photoisomers are proposed on the basis of NMR and UV spectroscopic evidences.*

Recently, a variety of aldose reductase inhibitors (ARI), to be effective for painful diabetic complications, cataract, retinopathy, nephropathy, and neuropathy, have been reported.[1-3] Among these inhibitors, epalrestat (ONO-2235) showed the potent inhibitory activity against ARs (e.g., IC_{50}s are 1.0 x 10^{-8}M and 2.6 x 10^{-8}M for ARs of rat lens and human placenta, respectively).[4] This compound is now in the stage of clinical study.

ONO-2235 is obtained as a sole product by condensation of rhodanin-N-acetic acid and α-methylcinnamaldehyde in acetic acid in the presence of sodium acetate,[5] and was considered to have the structure **3**.[4] However, despite of the fact that ONO-2235 has



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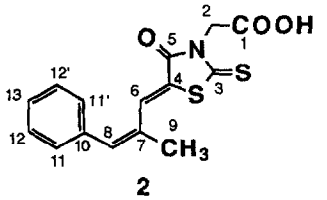

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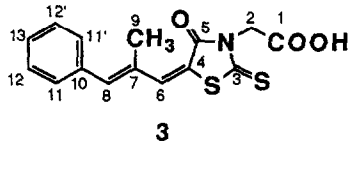

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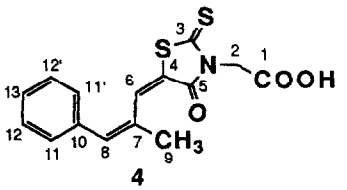

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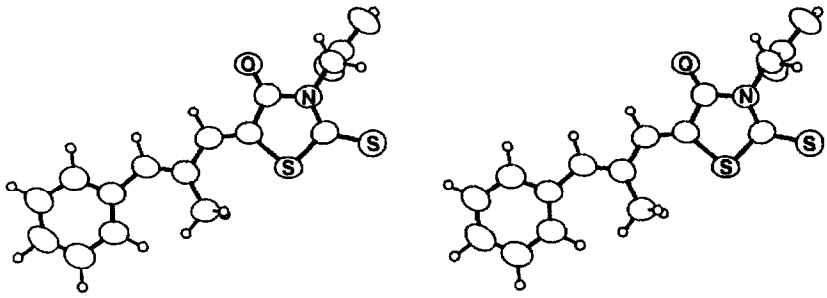

Fig. 1. A perspective drawing of ONO-2235 structure

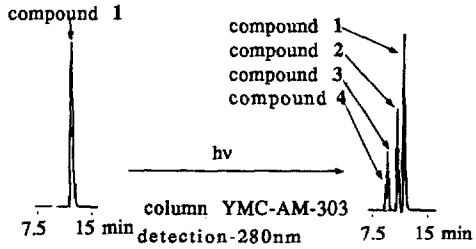

Fig. 2. HPLC analysis of photoisomerization of ONO-2235 **1**

Table 1. ^{13}C-NMR data of **1** and **2**
(acetone d_6, 125 MHz)

	Compound 1 (Hz)	Compound 2 (Hz)
1	167.32(t,6)	167.44(t,6)
2	45.32(t,145)	45.34(t,145)
3	194.46(t,5)	194.58(t,5)
4	121.85(b,s)	124.60(s)
5	167.49(dt,6,3)	167.77(dt,6,3)
6	140.41(dm,159)	133.55(dm,159)
7	134.20(q,7)	132.52(q,7)
8	144.43(dm,154)	146.27(dm,154)
9	16.36(qdd,125,6,8)	22.16(qt,125,7)
10	137.05(t,7)	137.32(t,7)
11,11'	129.34(dm,160)	129.50(dm,160)
12,12'	130.42(dm,160)	130.57(dm,160)
13	129.41(d,161)	129.37(dt,161,8)

Table 2. ^{13}C-NMR data of **5** and **6**
(acetone d_6, 125 MHz)

	Compound 5 (Hz)	Compound 6 (Hz)
1	167.26(t,6)	167.35(t,6)
2	45.15(t,142)	44.92(t,142)
3	194.77(t,5)	195.59(t,5)
4	127.06(m)	124.85(m)
5	165.71(dt,6,3)	165.38(dt,12,3)
6	140.24(dtt,159,5,7)	145.67(dtt,158,5,7)
7(10)	32.04(tm,125)	32.10(tm,128)
8	28.11(tm,126)	28.96(tm,122)
9	22.95(t,128)	22.97(tm,128)
10(7)	32.59(t,128)	32.59(tm,128)
11	14.14(q,123)	14.18(qm,123)

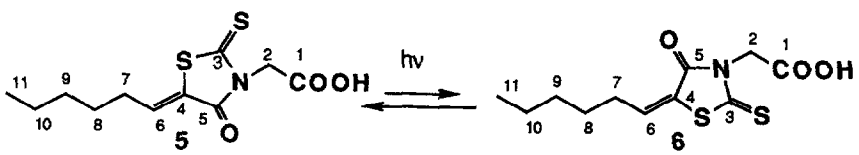

Fig. 3. Photoisomerization of model compound **5**

frequently been quoted as a key compound for discussion on the structure-activity-relationship, solid basis on the structure has not been published. Rigorous elucidation of the structure should provide very important information in designing more effective ARI.[6] We have recently established the structure **1** for this clinically important compound by X-ray crystallography. The result is described herein. Furthermore, ONO-2235, though stable in the dark, isomerizes very easily by photoirradiation even under a room light in solution (methanol, chloroform, acetone) to afford four isomers. We report also spectroscopic evidences which suggest the structures of all of these photoisomers.

Previous efforts to make crystals of ONO-2235 suitable for X-ray crystallography were fruitless because of their instability to X-ray radiation. Stable crystals of the compound containing equimolar ethanol were finally obtained by the recrystallization from ethanol and a single crystal X-ray diffraction study was performed on the crystal to culminate in the perspective drawing shown in Fig. 1.[7] It clearly demonstrates that ONO-2235 has the structure **1**, (*Z*)-3-carboxymethyl-[(2*E*)-methylphenylpropenylidene]-rhodanine. The compound **1** forms a large plane: the propenylidene chain is fully extended and is almost coplanar with the rhodanine part and phenyl ring. The dihedral angle between both rings is 6.5(3)°.

Structure of **1** being established, we paid attention to the structures of isomeric compounds **2**, **3**, and **4**, generated by the photoirradiation (fluorescent lamp) of **1** in methanol. The HPLC chart of the reaction mixtures is shown in Fig. 2. Since these photoisomers were very unstable, the structural study was very difficult. For example, isomers **3** and **4** are reisomerized to **1** and **2** even in the dark. The isolated **2** by means of preparative HPLC was also reisomerized to **1** during recrystallization from acetic acid. Careful NMR experiments on isolated **1** and **2** disclosed the following results.[8] (1) A significant NOE was observed for **1** between the methyl protons and the aromatic protons, but not between the methyl protons and C_8 proton, suggesting the *E* cofiguration for its C_7-C_8 double bond in accord with X-ray analysis. (2) In sharp contrast, a significant NOE was observed between the methyl protons and C_8 proton of **2**, but not between the methyl protons and aromatic protons. Thus the configuration of C_7-C_8 double bond of **2** was confirmed to be *Z* form. (3) Non-decoupled ^{13}C NMR spectra of **1** and **2** suggest that the configurations of C_4-C_6 double bonds in both compounds are identical, since in both cases C_5 carbons appeared as a similar double triplet pattern at δ 167.49 (J=6 and 3 Hz) and δ 167.77 (J=6 and 3 Hz), respectively, due to the long range coupling with C_6 and C_2 protons (Table 1). Thus the C_4-C_6 double bond of **2** was concluded to have *Z* configuration. This result was supported by the NMR study (Table 2) of a 3:1 mixture of model compounds **5** and **6**, obtained by the photoirradiation of **5**. Attempted separation of **5** and **6** failed since smooth reisomerization (**6** to **5**) occurred during separation. Although the C_5 carbon of **5** showed a signal at δ 165.71 (dt) with J=6 and 3 Hz, the same carbon signal of **6** appeared at δ 165.38 (dt) with J= 12 and 3 Hz.

The structural assignments of minor constituents **3** and **4** were particularly difficult because of their instability during the isolation. Smooth interconversion of

these compounds into **1** and **2** suggests that **3** and **4** are also configurational isomers of **1** around C_4-C_6 and/or C_7-C_8 double bond. We suggest structures for these compounds on the following basis. The ratio of **1** and **2** calculated by ^{1}H NMR was 5:3, but 3:1 from HPLC (detected by UV at 280 nm). This difference is rationalized by the different molar absorptivity of **1** and **2**, ε 7800 and 4500, respectively, at 280 nm in UV spectra and suggests that *Z,E* configuration of the double bonds, having larger ε, tends to give larger ratio in the mixture than Z,Z configuration. Now the ratio of the minor components **3** and **4** was 1:1 by ^{1}H NMR but 2:1 by HPLC. As the structural difference between **3** and **4** seems only in the configuration of C_7-C_8 double bond, the structures were assigned, accordingly. Thus **3** has *E, E* configuration and **4** *E, Z* configuration.

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7) The crystal data are as follows: $C_{15}H_{13}NO_3S_2 \cdot C_2H_5OH$, Mw=365.46, triclinic, space group P1, Z=2, cell constants a=14.968(3), b=100.82(2), c=8.403(2) Å, α=119.80(2), β=100.82(3), γ=75.09(2)°, V=897.3(4) Å^3, D_m=1.365(3)g.cm^{-3}, D_x=1.353g.cm^{-3}, μ(Cu Kα)=28.17 cm^{-1}. A total of 3052 reflections was measured within 2θ=130°. The crystal structure was determined by the direct method, and atomic parameters were refined by block-diagonal least-squares calculations. The final *R* value was 0.093 for 2157 reflections(I ≥3σ(I)) including 19 hydrogen atoms, for which isotropic thermal parameters were used. The final atomic parameters, bond lengths and angles will be deposited with the Cambridge Crystallographic Data Center.
8) ^{1}H-NMR data (500 MHz, methanol d_4): compound **1** (ONO-2235) δ 2.31(3H, d, 1.5Hz), 4.88 (2H, s), 7.39 (1H, tt, 7.5, 1 Hz) 7.47 (2H, m), 7.52 (2H, m), 7.60(1H, d, 1 Hz); compound **2** 2.28 (3H, d, 1.5 Hz), 4.83(2H, s), 7.18(1H, b.s), 7.33-7.46 (5H, m), 7.80 (1H, d, 1 Hz). The ^{1}H-NMR spectrum of a mixture by photoisomerization showed the presence of other 2 sets: one is 2.30 (b.d), 6.87, and 7.05 and the other is 2.33 (d), 7.13, and 7.16. These sets are corresponding to the compounds **3** and **4**.

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