

TA-3037A, A NEW INHIBITOR OF GLUTATHIONE *S*-TRANSFERASE,  
PRODUCED BY ACTINOMYCETES

## II. STRUCTURE DETERMINATION

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TA-3037A, a new inhibitor of glutathione *S*-transferase, has been isolated from the culture broth of *Streptomyces* sp. TA-3037. The structure of TA-3037A was defined as (*Z*)-3-benzylidene-3,4-dihydro-2-oxo-2*H*-1,4-benzoxazine-5-carboxylic acid by an analysis of spectral properties and chemical studies of TA-3037A and its derivatives.

In the preceding paper<sup>1</sup>) we have described the isolation, purification and the biological properties of TA-3037A (**1**), a novel inhibitor of glutathione *S*-transferase. In this paper, we wish to describe the structure of **1**. **1** was obtained as yellow crystals. The UV and IR spectra of **1** are shown in Figs. 1 and 2, respectively. The UV spectra showed absorption maxima at 204 (log  $\epsilon$  4.43), 242 (4.20), 282 (3.97) and 383 nm (4.16) in EtOH, 205 (log  $\epsilon$  4.34), 241 (4.26), 282 (3.96), 350 (sh, 3.94) and 382 nm (4.16) in 0.03 M HCl-EtOH, and 324 nm

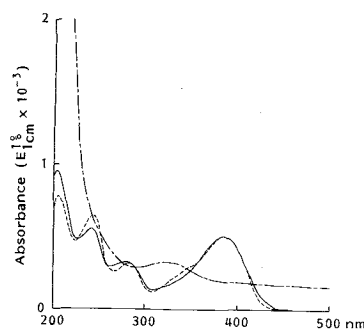
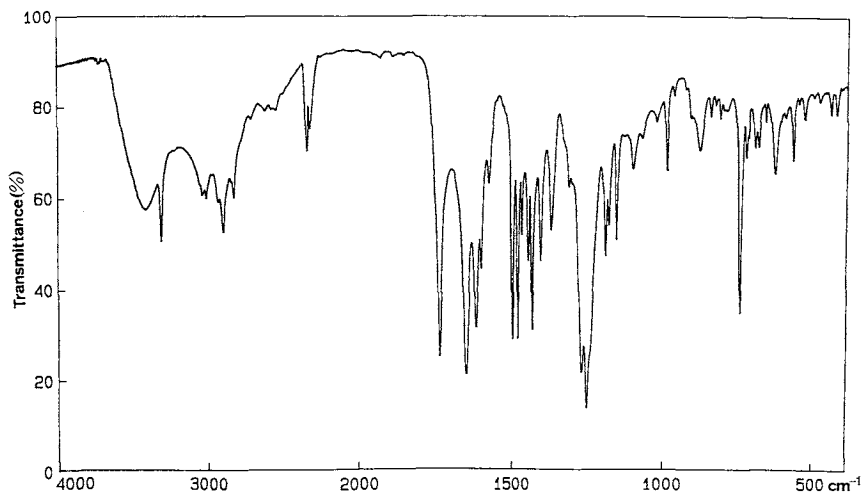
Fig. 1. UV spectra of TA-3037A (**1**).— Neutral EtOH, - - - acidic EtOH,  
- · - · - alkaline EtOH.Fig. 2. IR spectrum of TA-3037A (**1**).

Table 1.  $^1\text{H}$  NMR data<sup>a</sup> for TA-3037A (1) and its methyl ester (2).

Proton	TA-3037A (1)	TA-3037A methyl ester (2)
4-NH	11.10 (1H, br s)	10.66 (1H, br s)
6-H	7.66 (1H, dd, $J=8.0, 1.5$ )	7.72 (1H, dd, $J=8.0, 1.5$ )
7-H	6.90 (1H, t, $J=8.0$ )	6.82 (1H, t, $J=8.0$ )
8-H	7.32 (1H, br dd, $J=8.0, 1.5, <1.0$ )	7.21 (1H, dd, $J=8.0, 1.5, 0.5^*$ )
9-COOH	8.85 (1H, br s)	
10-H	6.65 (1H, s)	6.86 (1H, s)
12-H, 16-H	7.60 (2H, d, $J=8.0$ )	7.58 (2H, d, $J=8.0$ )
13-H, 15-H	7.46 (2H, t, $J=8.0$ )	7.50 (2H, t, $J=8.0$ )
14-H	7.34 (1H, t, $J=8.0$ )	7.34 (1H, t, $J=8.0$ )
17-COOCH <sub>3</sub>		3.93 (3H, s)

<sup>a</sup> 400 MHz; chemical shifts in ppm, coupling constants in Hz. TA-3037A was measured in DMSO-*d*<sub>6</sub> and its methyl ester was in CDCl<sub>3</sub>.

\* Obtained by resolution enhancement method.

Table 2.  $^{13}\text{C}$  NMR data<sup>a</sup> for TA-3037A (1) and its methyl ester (2).

Carbon	TA-3037A (1)	TA-3037A methyl ester (2)
C-2	157.3 (s)	158.0 (s)
C-3	123.3 (s)	122.7 (s)
C-4a	129.9 (s)	130.3 (s)
C-5	112.0 (s)	111.3 (s)
C-6	126.7 (d)	126.9 (d)
C-7	118.7 (d)	118.6 (d)
C-8	120.4 (d)	121.0 (d)
C-8a	140.3 (s)	140.5 (s)
C-9	169.3 (s)	167.6 (s)
C-10	109.4 (d)	112.1 (d)
C-11	134.0 (s)	134.1 (s)
C-12, C-16	128.0 (d)	128.2 (d)
C-13, C-15	129.0 (d)	129.2 (d)
C-14	127.8 (d)	128.1 (d)
C-17		52.3 (q)

<sup>a</sup> 100 MHz; chemical shifts in ppm. TA-3037A was measured in DMSO-*d*<sub>6</sub> and its methyl ester was in CDCl<sub>3</sub>.

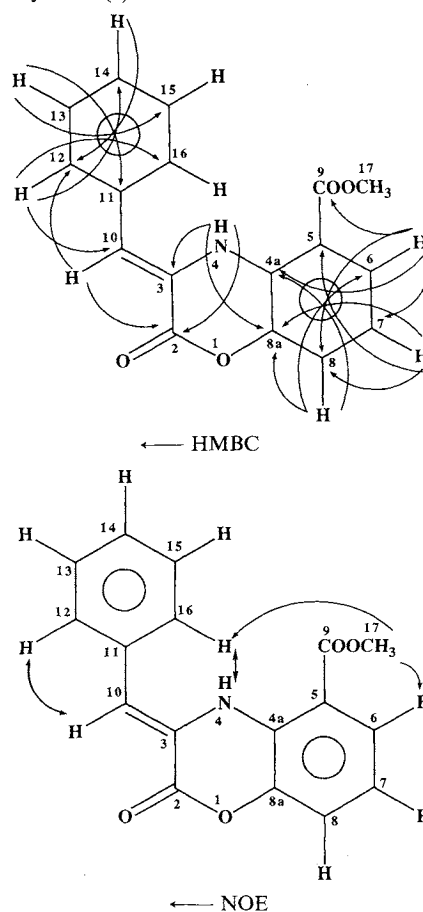
(3.96) in 0.03 M NaOH - EtOH.

The IR spectrum (KBr) showed the absorption of the hydroxyl ( $3440\text{ cm}^{-1}$ ), N-H ( $3340\text{ cm}^{-1}$ ), carboxyl or lactone ( $1752\text{ cm}^{-1}$ ) and aromatic derivative ( $1600, 1507$  and  $1439\text{ cm}^{-1}$ ). The molecular formula of **1** was determined to be C<sub>16</sub>H<sub>11</sub>NO<sub>4</sub> by HRFAB-MS data ( $m/z$  280.0607 (M-H)<sup>-</sup>).

For an elucidation of the structure<sup>2)</sup>, **1** was converted to a methyl ester derivative (**2**) by treatment with trimethylsilyl diazomethane.

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of **1** and **2** are presented in Tables 1 and 2. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of **1** and **2** suggested the presence of benzylidene group and 1,2,3-trisubstituted benzene

Fig. 3. HMBC and NOE experiments of TA-3037A methyl ester (2).



ring. The partial structure was supported by acid hydrolysis of **1** to give 3-hydroxyanthranilic acid.

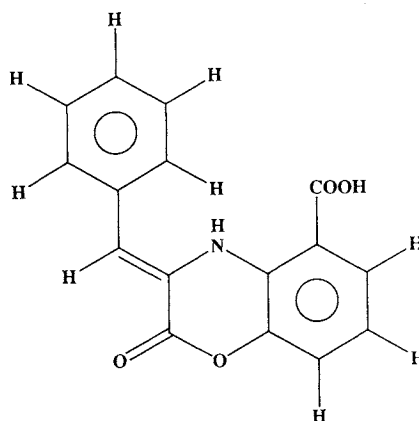
The broad singlet corresponding to a carboxyl proton was observed at  $\delta$  8.85 in the  $^1\text{H}$  NMR spectrum of **1**, while in that of **2** this peak disappeared and one methoxyl peak appeared at  $\delta$  3.93 ( $\text{COOCH}_3$ ).

The positional arrangement of one carbonyl group, one oxygen atom and one nitrogen atom in the heterocyclic ring of **2** was defined by  $^1\text{H}$ -detected heteronuclear multiple-bond correlation (HMBC) spectra and nuclear Overhauser effect (NOE) experiments (Fig. 3). 10-H ( $\delta$  6.86) coupled with C-2 ( $\delta$  158.0), demonstrating the arrangement from C-10 to C-2.

Amine proton ( $\delta$  10.66) coupled with C-2 ( $\delta$  158.0), indicated the arrangement from N-4 to C-2. Amine proton ( $\delta$  10.66) also coupled with C-8a ( $\delta$  140.5) indicated the connectivity from N-4 to C-8a, which was supported by the observation of a long range coupling ( $^5J_{\text{NH},8\text{-H}}=0.5\text{ Hz}$ ) between amine proton ( $\delta$  10.66) and 8-H ( $\delta$  7.21). The observation of NOE between amine proton ( $\delta$  10.66) and 12-H (16-H,  $\delta$  7.58), and also between methoxyl proton ( $\delta$  3.93) and 12-H (16-H,  $\delta$  7.58) showed *cis* configuration of the double bond (between C-3 and C-10).

Thus, the structure of **1** was determined to be (*Z*)-3-benzylidene-3,4-dihydro-2-oxo-2H-1,4-benzoxazine-5-carboxylic acid (Fig. 4).

Fig. 4. Structure of TA-3037A (**1**).



## Experimental

### Methylation of **1**

A solution of trimethylsilyl diazomethane in hexane was added to a solution of 5 mg of **1** in the mixture of 1 ml  $\text{CHCl}_3$  and 1 ml MeOH, and this was allowed to stand for 1 minute at room temperature. Excess reagent and solvents were removed under reduced pressure. **1**: Rf 0.60, **2**: Rf 0.77 (Silica gel TLC plate, Merck Art. 5715, BuOH-MeOH- $\text{H}_2\text{O}$ , 4:1:2) **2**: FAB-MS,  $m/z$  296 ( $\text{M}+\text{H}$ ) $^+$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR data are presented in Tables 1 and 2, respectively.

### Hydrolysis of **1**

Conc HCl (12N, 250  $\mu\text{l}$ ) was added to a solution of **1** (2 mg) in 250  $\mu\text{l}$   $\text{CH}_3\text{COOH}$  and heated for 5 hours at 110°C in a sealed tube. The solution was concentrated to dryness. The main product was compared with the standard, 3-hydroxyanthranilic acid. Rf value of 3-hydroxyanthranilic acid was 0.72 (Silica gel TLC plate, Merck Art. 5715, BuOH-MeOH- $\text{H}_2\text{O}$ , 4:1:2).

## References

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