Chem. Pharm. Bull. 19(11)2289—2293(1971)

UDC 547.892.02.04 : 615.332.011.5

The Structures of Tomaymycin and Oxotomaymycin[1]

Kazuo Kariyone, Hisatoyo Yazawa and Masanobu Kohsaka

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd.[2]

(Received April 12, 1971)

Tomaymycin (2) and oxotomaymycin (3) are antibiotics isolated from *Streptomyces achromogenes* var. *Tomaymycetics*.

The structure of tomaymycin (2) was determined by degradation reactions together with spectral analysis and by synthesis of the important derivative (**8b**) containing the basic skeleton of the antibiotic. The structure of oxotomaymycin (3) was also established by chemical correlation to the derivative (**7a**) of tomaymycin.

Tomaymycin, a new antitumor antibiotic was isolated from *Streptomyces achromogenes* var. *Tomaymycetics* by Arima, *et al.*[3]

In the previous paper of this series,[4] the structure of tomaymycin was proposed as ethylidene-5,10,11,11a-tetrahydro-8-hydroxy-7,11-dimethoxy-5-oxo-1H-pyrrolo-(2,1-C)(1,4)-benzodiazepine (**1**). The structure of the part A was unambigously established by obtaining 4-ethoxy-5-methoxyanthranilic acid (**4**), which was formed by alkaline hydrolysis of ethyl ether of tomaymycin. Though the structure of the remaining part (part B) of tomaymycin was deduced from spectral data of tomaymycin and its derivatives, no conclusive evidence was presented in the previous paper.[4]

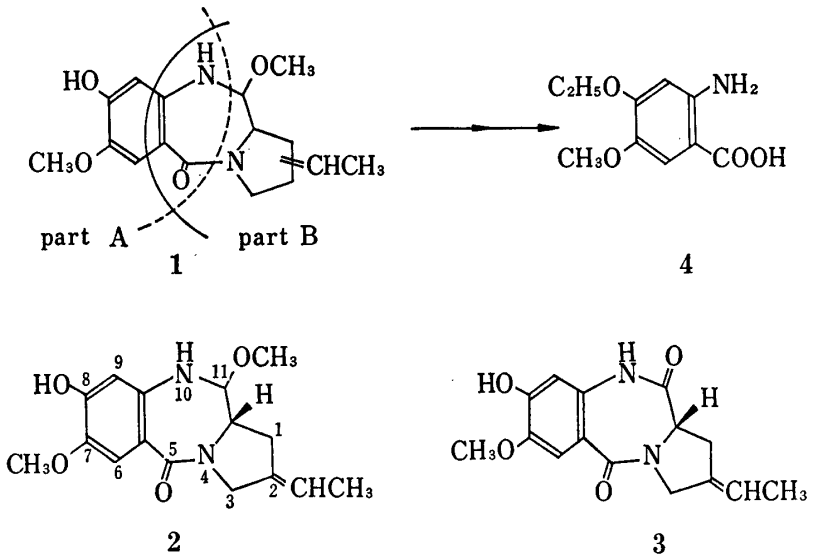


Chart 1

This report deals with identification of an important derivative (**8b**) by synthesis, establishment of the complete structure of tomaymycin by chemical degradation, and determination of the structure of oxotomaymycin by correlation to a derivative (**7a**) of tomaymycin.

1) Structure of tomaymycin II.
2) Location: *1, Kashimacho, Higashiyodogawa-ku, Osaka.*
3) Isolation of tomaymycin, K. Arima, M. Kohsaka, G. Tamura, H. Imanaka and H. Sakai. *J. Antibiotics*, in press.
4) Structure of tomaymycin I, M. Kohsaka, K. Arima, G. Tamura, H. Imanaka and H. Sakai. *J. Antibiotics*, in press.

Since there was no information concerning the position of the ethylidene group on the pyrrolidine ring of tomaymycin in the previous paper,[4] it is necessary to solve this problem at first.

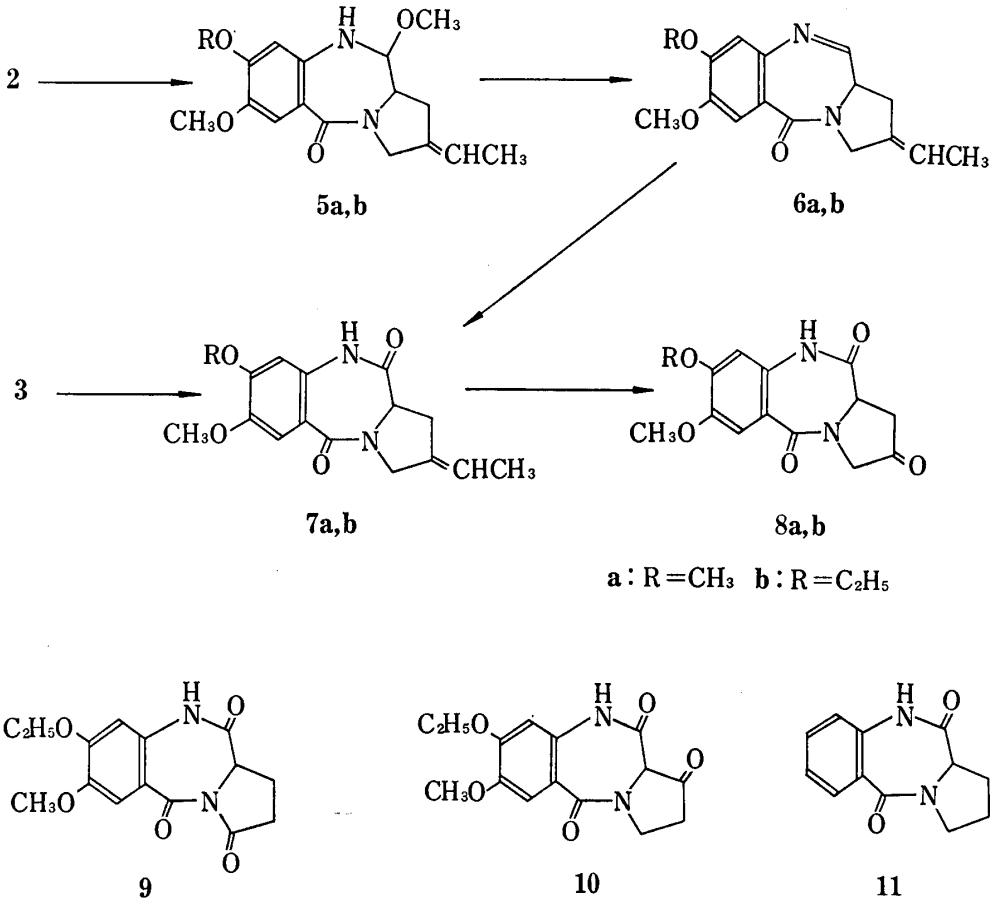


Chart 2

By treatment with diazomethane, tomaymycin (**2**) gave the methyl ether (**5a**), which, on heating under reduced pressure, afforded the desmethanol product (**6a**) as pale yellow powder.[4] Oxidation of the desmethanol derivative (**6a**) to oxotomaymycin methyl ether (**7a**) could be effected by various reagent such as manganese dioxide, chromium trioxide–pyridine, and *m*-chloroperbenzoic acid: among the oxidizing reagent examined *m*-chloroperbenzoic acid gave the best result.

The corresponding ethyl ether (**7b**) was also prepared from tomaymycin (**2**).

Ozonolysis of oxotomaymycin ethyl ether (**7b**) in methanol at −76°, followed by reduction of the resulting hydroperoxide with dimethyl sulfide[5] gave a trioxo compound (**8b**), for which three posible structures (**8b**, **9**, **10**) are conceivable.

The infrared (IR) spectrum of **8b** showed three carbonyl bands: a C-5 amide carbonyl (1610 cm^{-1}), a C-11 amide carbonyl (1700 cm^{-1}) and a newly appeared carbonyl (1775 cm^{-1}) due to a 5-membered ring ketone. The nuclear magnetic resonance (NMR) spectrum of **8b** showed characteristic signals as follows: a C-11a proton (1H, quartet, J=4 and 9 Hz, τ 5.42), a C-1 proton (2H, multiplet, τ 5.80—6.20) and a C-3 proton (2H, multiplet, τ 6.80—7.25).

These IR and NMR spectral data suggest that the structure of the trioxo compound must be **8b**, eliminating two other possibilities (**9** and **10**).

5) J. J. Pappas, W. P. Keaveny, E. Gancer and M. Berger, *Tetrahedron Letters*, **1966**, 4273.

Further the structure of the trioxo compound (**8b**) was unambiguously established by the synthesis as illustrated in Chart 3.

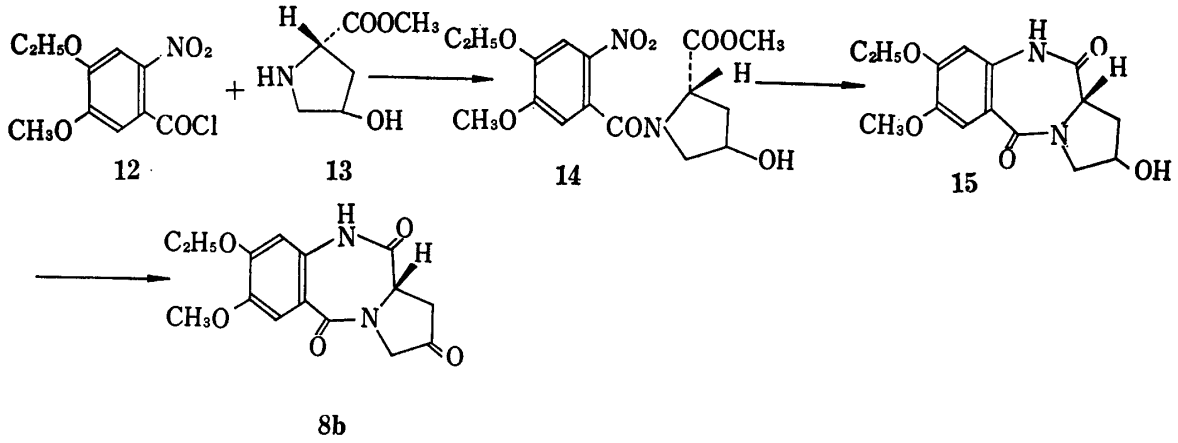


Chart 3

Methyl 1-(2-nitro-4-ethoxy-5-methoxybenzoyl)-*l*-hydroxyprolinate (**14**) was obtained from 2-nitro-4-ethoxy-5-methoxybenzoyl chloride (**12**)[6] and methyl *l*-hydroxyprolinate (**13**)[7] by Schotten–Baumann reaction. Catalytic hydrogenation of the proline ester (**14**) over palladium-charcoal afforded a dark yellow oil, which was cyclized by heating in toluene to give a hydroxy dioxo compound (**15**). Oxidation of **15** was conveniently achieved by Jones reagent in acetone–DMF to give a trioxo compound (**8b**).

The synthetic trioxo compound was found to be identical with the natural trioxo compound (**8b**) derived from tomaymycin (**2**) by comparison of spectral properties (IR, ultraviolet (UV), NMR, and mass), the specific rotations, and the melting points.

Since it is now evident that the ethylidene group is attached to the C-2 position, considering the spectral properties of tomaymycin (**2**) and its derivatives, the structure of tomaymycin was established as shown in **2**, including the absolute configuration of the C-11a position, which is same as that of *l*-hydroxyproline.

It is known that the oxaziranes undergo two types of thermal rearrangement, depending on the nature of substituents:[8] while aryl-substituted oxaziranes isomerize at elevated temperatures to nitrones, alkyl-substituted ones give amides.

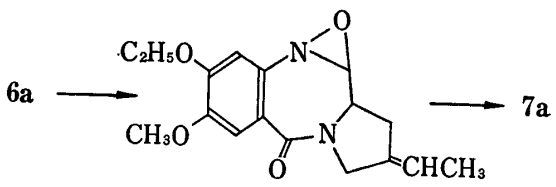


Chart 4

It was interesting that the oxazirane derivatives (**16**) of desmethanol compound (**6a**) rearranged readily into an amide (**7a**) even at room temperature.

A minor product named oxotomaymycin was isolated from the same fermentation broth of tomaymycin. The molecular formula was determined as $C_{15}H_{16}O_4N_2$ by high resolution mass spectral analysis. The IR spectrum of oxotomaymycin (**3**) showed bands in the range between 3300 and 3400 cm^{-1} due to hydroxy and amide (NH) group and two bands at 1685 and 1610 cm^{-1} due to amide carbonyl group. The NMR spectrum showed signals due to aromatic protons at 2.76 τ (1H, singlet) and 3.40 (1H, singlet), a methoxy group at 6.20 τ

6) S. Uyeo and N. Yanaihara, *J. Chem. Soc.*, **1959**, 172.
7) They were obtained by treating dry *l*-hydroxyproline–HCl with absolute methanol.
8) W.D. Emons, *J. Am. Chem. Soc.*, **78**, 6208 (1956); W.D. Emons, *ibid.*, **79**, 5739 (1957); H. Krinm, *Ber.*, **90**, 2184 (1957).

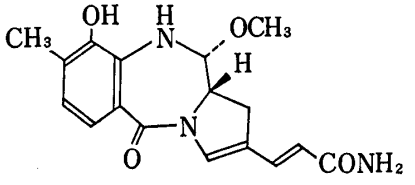
(3H, singlet) and the methyl of an ethylidene group at 8.33 τ (3H, broad doublet, J=*ca.* 7 Hz). The mass spectrum was very similar to that of the dioxo compound (**11**).

On the basis of these spectral data, the structure of oxotomaymycin was deduced to be 5,10,11,11a-tetrahydro-8-hydroxy-7-methoxy-2-ethylidene-5,11-dioxo-1H-pyrrolo-(2,1-C)(1,4)-benzodiazepine. The inference was confirmed by chemical correlation of oxotomaymycin (**3**) with tomaymycin: oxotomaymycin was converted to the methyl ether by diazomethane, which was identical with the compound (**7a**) derived from tomaymycin (**2**).

Tomaymycin exhibits a strong inactivating effect against various bacteriophages and animal viruses but oxotomaymycin does not show such activities.

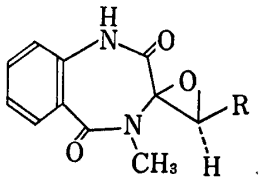
There are few natural products so far known, having 1,4-benzodiazepine as basic skelton: anthramycin (**17**),[9] cyclopenin (**18a**) and cyclopenol (**18b**).[10]

Now tomaymycin and oxotomaymycin are added to this group.



anthramycin

17



cyclopenin R : C_6H_5– (18a)

cyclopenol R : p-OH-C_6H_4– (18b)

Chart 5

Experimental[11]

Isolation of Oxotomaymycin (3)——To the culture filtrate, 0.3% active carbon was added. Oxotomaymycin was completely adsorbed on the carbon and then eluted with the following mixed solvent system; MeOH: C_5H_5N: 28% NH_4OH: H_2O=86: 3: 1: 10.

The eluate from the carbon was concentrated and the resulting oily material was dissolved in H_2O. Oxotomaymycin was extracted with $CHCl_3$ from the aqueous solution after acidification by HCl to pH 3.0. The chloroform extract was concentrated and chromatographed on silicic acid with EtOAc. The eluate was concentrated to dryness *in vacuo* and the residual yellowish powder was dissolved in a small amount of MeOH. Crystals of tomaymycin were obtained by keeping the solution in a refrigerator overnight. After the mother liquor was kept in a refrigerator for 2 or 3 days, oxotomaymycin was obtained as crystals, mp 230° (decomp.). Oxotomaymycin thus obtained was still contaminated with a small amount of tomaymycin and could not be obtained in the completely pure state free from tomaymycin. IR ν_{max} cm^{-1}: 3400, 3300, 1685, 1635, 1610. NMR (d_6-DMSO) τ: 2.76 (1H, s), 3.40 (1H, s), 4.60 (1H, m), 6.20 (3H, s), 8.33 (3H, br. d, J=*ca.* 7 Hz). The high resolution mass spectrum indicated a molecular weight of 288 and a formula of $C_{15}H_{16}O_4N_2$ (Calcd: 288.1122. Found: 288.1110).

Oxotomaymycin Methyl Ether (7a)——1) From Tomaymycin (2): *m*-Chloroperbenzoic acid (1.05 g, 0.006 mol) in CH_2Cl_2 (10 ml) was added dropwise to a solution of the desmethanol product (**6a**)[4] (1.23 g) in CH_2Cl_2 (20 ml) at −20° and the temperature of the mixture was gradually raised to room temperature. After stirring at room temperature for 4 hr, the mixture was filtered to remove insoluble material. The filtrate was washed with sat. $KHCO_3$ aq and dried over $MgSO_4$. On removal of solvent, brown-yellow oil was obtained. The oil was crystallized from acetone to give 0.26 g (20% yield) of colorless crystals. Recrystallization from methylcellosolve gave the dioxo compound (**7a**), mp 279—281°. IR ν_{max} cm^{-1}: 3260, 1680, 1640, 1615. NMR (d_6-DMSO) τ: 2.75 (1H, s), 3.28 (1H, s), 4.50 (1H, m), 6.20 (6H, s), 5.5—6.0 (3H, m), 6.7—7.5 (2H, m),

9) W. Leimgruber, V. Stefanovic, F. Schenker, A. Karr and J. Berger, *J. Am. Chem. Soc.*, **87**, 5791 (1965); W. Leimingruber, A.D. Batcho and F. Schenker, *ibid.*, **87**, 5793 (1965).

10) Y.S. Mohammed, *Tetrahedron Letters*, **1963**, 1953.

11) All melting points were uncorrected. The infrared spectra were recoreded in nujol mulls with a Hitachi EPI S_2 spectrophotometer. The nuclear magnetic resonance spectra were measured with a Varian A-60 spectrometer using tetramethylsilane as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). The mass spectra were determined with a Nihondenshi JMS-01 SG spectrometer.

8.32 (3H, br. d, J=*ca.* 7 Hz). *Anal.* Calcd. for $C_{16}H_{18}O_4N_2$: C, 63.56; H, 6.00; N, 9.27. Found: C, 63.09; H, 6.00; N, 9.16. Mass Spectrum: M^+=302.

2) From Oxotomaymycin (**3**): To a solution of oxotomaymycin (0.5 g) in MeOH (20 ml) was added an etheral solution of diazomethane and the mixture was kept at room temperature overnight. The solution was evaporated and the residue was extracted with CH_2Cl_2. The organic layer was washed with H_2O, dried over $MgSO_4$, and evaporated. The crude product was purified by recrystallization from methylcellosolve to give 0.23 g of the dioxo compound (**7a**), mp 278—280°. IR ν_{max} cm^{-1}: 3260, 1680, 1640, 1615. Mass Spectrum: M^+=302.

Oxotomaymycin Ethyl Ether (7b)——To a solution of oxotomaymycin (1.8 g) in DMF (20 ml) an etheral solution of diazoethane was added, and the reaction mixture was allowed to stand overnight at room temperature. A small amount of HOAc was added to decompose excess diazoethane. After evaporation of solvent, the residue was extracted with CH_2Cl_2. The organic layer was washed with H_2O, and dried over K_2CO_3. Removal of solvent gave a white solid (2.5 g), which was recrystallized from MeOH, mp 253—254°. IR ν_{max} cm^{-1}: 3220, 1680, 1615, 1520. NMR (C_5D_5N) τ: 0.30 (1H, m, NH), 2.18 (1H, s), 3.14 (1H, s), 6.60 (1H, m), 5.20 (1H, m), 5.40—5.75 (2H, m), 5.95 (2H, q, J=8 Hz), 6.20 (3H, s), 6.20—6.50 (1H, m), 7.10—7.65 (1H, m), 8.46 (3H, br. d, J=7 Hz), 8.70 (3H, t, J=8 Hz). *Anal.* Calcd. for $C_{17}H_{20}O_4N_2$: C, 64.54; H, 6.36; N, 8.86. Found: C, 64.42; H, 6.23; N, 8.84. Mass Spectrum: M^+=316.

Ozonolysis of Oxotomaymycin Ethyl Ether (7a)——Ozone was passed through a solution of oxotomaymycin ethyl ether (**7a**, 0.59 g) in MeOH (250 ml) for 5 min at −76°. The starting material was nomore detected by thin-layer chromatograpy (TLC) ($CHCl_3$: MeOH=10:1 as solvent). The solution was flushed with N_2, to which dimethylsulfide (1.5 ml) was added. The solution was then stirred at 10—0° for 1 hr and stirred at room temperature for 1 hr. The solvent was removed *in vacuo*, and the mixture was extracted with CH_2Cl_2. The solution was washed with H_2O and dried over $MgSO_4$. On removal of solvent *in vacuo* there remained a pale yellow solid, which was separated by preparative TLC ($CHCl_3$: MeOH=10:1 as solvent) to yield trioxo compound (**8b**) as colorless crystals (0.12 g). The pure sample was recrystallized from 95% EtOH, mp 233—235° (decomp). IR ν_{max} cm^{-1}: 3250, 3200, 1775, 1700, 1635, 1610. UV λ_{max}^{MeOH}: 235 nm (ε =36000). NMR (d_6-DMSO) τ: −4.0 (1H, br. s, NH), 2.75 (1H, s), 3.30 (1H, s), 5.42 (1H, dd, J=4 and 9 Hz), 5.80—6.20 (2H, m), 6.00 (2H, q, J=8 Hz), 6.20 (3H, s), 6.80—7.25 (2H, m), 8.63 (3H, t, J=8 Hz). α_D^{22}: +817° (MeOH). *Anal.* Calcd. for $C_{15}H_{16}O_5N_2$: C, 59.20; H, 5.30; N, 9.21. Found: C, 59.04; H, 5.23; N, 9.04.

Methyl 1-(2-Nitro-4-ethoxy-5-methoxybenzoyl)-*l*-hydroxyprolinate (14)——A solution of methyl *l*-hydroxyprolinate–HCl (3.1 g, 0.017 mol) and Et_3N (3.9 g, 0.038 mol) in CH_2Cl_2 (80 ml) was strirred vigorously at −10° while 2-nitro-4-ethoxy-5-methoxybenzoyl chloride (4.5 g, 0.017 mol) in CH_2Cl_2 (20 ml) was added slowly. After stirring for 2 hr at room temperature H_2O was added to the solution. The organic layer was separated and washed with 10% HCl and H_2O, and dried over $MgSO_4$. On removal of solvent there was obtained as amorphous solid (4.8 g). Mass Spectrum: M^+=324. IR ν_{max} cm^{-1}: 3400, 1745, 1635, 1580, 1520, 1340. NMR ($CDCl_3$) τ: 2.33 (1H, s), 3.22 (1H, s), 5.40—6.25 (3H, m, C**H**$_2$, C**H**), 6.00 (3H, s), 6.60—6.95 (2H, m), 7.85—8.25 (4H, m), 8.46 (3H, t, J=8 Hz).

2,3,5,10,11,11a-Hexahydro-7-methoxy-8-ethoxy-5,11-dioxo-2-hydroxy-1H-pyrrole-(2,1-C) (1,4)-benzodiazepine (15)——A solution of the proline derivative **14** (4.5 g, 0.013 mol) in MeOH (150 ml) was hydrogenate in the presence of 10% palladium charcoal (1 g) at atmospheric pressure. The catalyst was removed by filtration and the filtrate was evaporated to yield pale yellow oil (4 g), which was dissolved in toluene (100 ml) and refluxed for 1 hr. The residue obtained by evaporation of solvent was recrystallized from 95% EtOH to give 2.5 g (70% yield) of **15**, mp 248—249° (decomp). IR ν_{max} cm^{-1}: 3450, 3380, 3280, 3220, 1675, 1610, 1605. NMR (d_6-DMSO) τ: −0.15 (1H, br. s, NH), 2.67 (1H, s), 3.25 (1H, s), 4.80 (1H, br. s, OH), 5.50—6.10 (4H, m, C**H**$_2$, C**H**, C**H**-OH), 6.20 (3H, s), 6.40—6.75 (2H, m), 7.1—7.50 (1H, m), 7.80—8.30 (1H, m), 8.65 (3H, t, J=8 Hz). α_D^{22}: +1200° (MeOH). *Anal.* Calcd. for $C_{15}H_{18}O_5N_2$: C, 58.81; H, 5.92; N, 9.15. Found: C, 58.65; H, 5.80; N, 8.93.

2,3,5,10,11a-Hexahydro-7-methoxy-8-ethoxy-2,5,11-trioxo-1H-pyrrolo(2,1-C)(1,4)-benzodiazepine (8b)——To a solution of the benzodiazepine derivative **15** (1.8 g, 0.0057 mol) in DMF (10 ml) and acetone (100 ml) was added with stirring Jones reagent (3.6 ml)[12] at room temperature. After stirring for 15 min the mixture was evaporated *in vacuo*, and diluted with H_2O. The resulting mixture was extracted with CH_2Cl_2 and the organic layer was washed with H_2O, sat. $NaHCO_3$ aq., and H_2O, and dried over $MgSO_4$. Removal of solvent gave a solid (0.75 g), which was chromatographed on silicic acid (20 g) with $CHCl_3$–MeOH (10:1). The crude product obtained was recrystallized from 95% EtOH to give (0.4 g) of trioxo compound (**8b**), mp 228—230° (decomp). IR ν_{max} cm^{-1}: 3250, 3200, 1775, 1700, 1635, 1610. UV λ_{max}^{MeOH}: 235 nm (ε=35,500). NMR (d_6-DMSO) τ: −0.40 (1H, br. s, NH), 2.75 (1H, s), 3.30 (1H, s), 5.42 (1H, dd, J=4 and 9 Hz), 5.80—6.15 (2H, m), 6.0 (2H, q, J=8 Hz), 6.20 (3H, s), 6.80—7.20 (2H, m), 8.63 (3H, t, J=8 Hz). α_D^{22}: +814° (MeOH). *Anal.* Calcd. for $C_{15}H_{16}O_5N_2$: C, 59.20; H, 5.30; N, 9.21. Found: C, 59.23; H, 5.25; N, 9.32.

12) Chromiun trioxide reagent: to a solution of chromium trioxide (2.6 g) in H_2O (10 ml) was added conc. H_2SO_4 (2.3 ml).