Chemical Modification of Tylosin

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(Received for publication October 18, 1994)

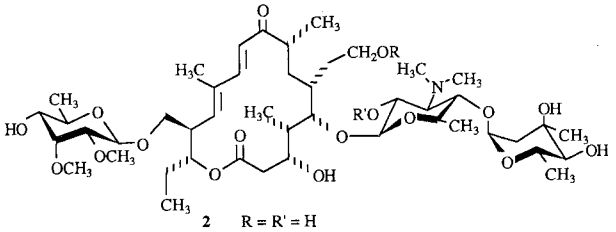
20-Homo tylosin, 20-nor tylosin, 20-deoxy-19,20-didehydro tylosin (6-vinyl tylosin) and 2″,3″-didehydro-3″-deoxy tylosin were prepared by multi-step procedures. 20-Homo tylosin was about twice less active than tylosin while the other coumpounds exhibited very weak antibacterial activity.

In view of the continuing considerable interest in 16-membered macrolide antibiotics[1], we decided to explore further the chemistry of tylosin (**1**). This macrolide has a two-carbon side chain of crucial biological importance under the form of an acetaldehyde. We were interested in performing basic modifications to the side-chain by both lengthening and shortening it by one carbon atom. Thus, our major target molecules were 20-homo tylosin (**8**) and 20-nor tylosin (**12**).

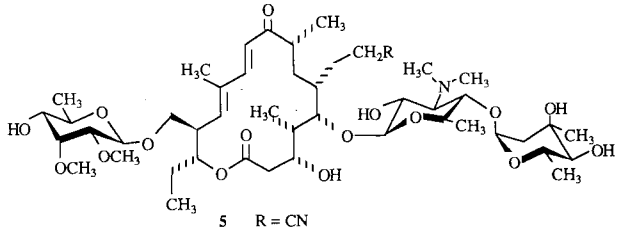
Results and Discussion

A straightforward approach to the synthesis of 20-homo tylosin (**8**) was attempted from the known 20-*O*-tosyl relomycin (**3**)[2] by potassium cyanide treatment and subsequent DIBAL mediated regiospecific reduction of the intermediate 20-*C*-cyano-20-deoxy relomycin (**5**). The latter could be prepared in 44% yield when 2′-*O*-acetyl-20-*O*-tosyl relomycin (**4**) was treated overnight at 60°C by two equiv. of potassium cyanide in acetonitrile in the presence of 2 equiv. of crown ether and under vigorous stirring (sonication), followed by methanol induced 2′-de-*O*-acetylation.

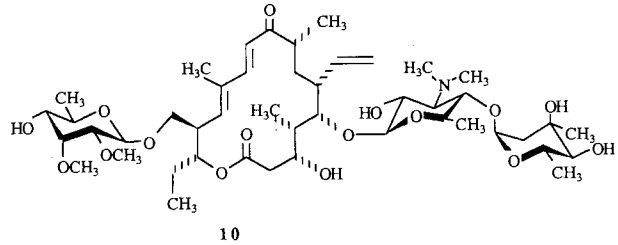
However, surprisingly, DIBAL treatment of **5** under a variety of conditions led only to the recovery of the starting 20-*C*-cyano compound. Therefore, as a new approach towards 20-homo tylosin (**8**), we decided to investigate a strategy based on the reaction of α,β-epoxy sulfones with hydride ion[3] for the chain elongation process. Carbonyl compounds have been reported to



2 R = R′ = H
3 R = Ts ; R′ = H
4 R = Ts ; R′ = Ac



5 R = CN
7 R = CH_2OH
8 R = CHO



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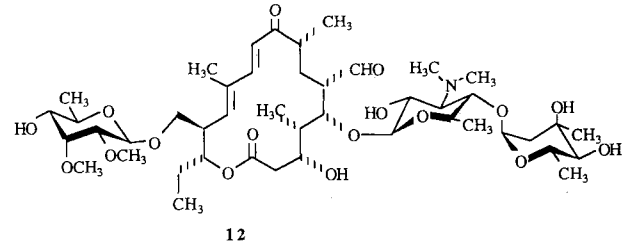

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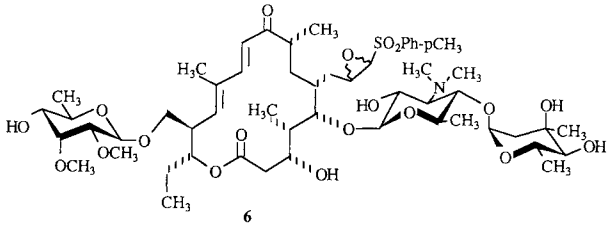
This paper is dedicated to Professor SATOSHI ŌMURA, President of Kitasato Institute, Tokyo, on the occasion of his 60th birthday, for his immense contribution to the study of antibiotics.



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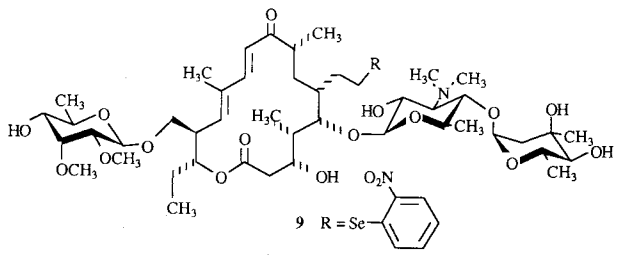
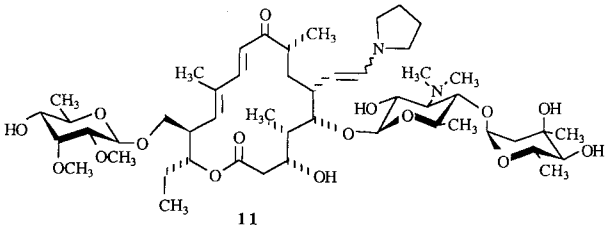


9 R = Se



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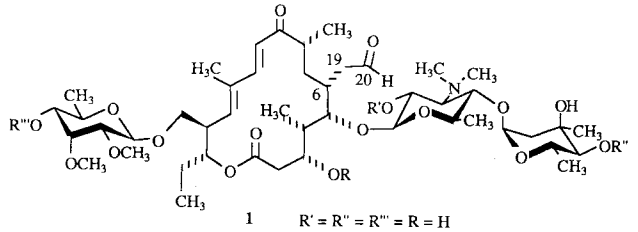
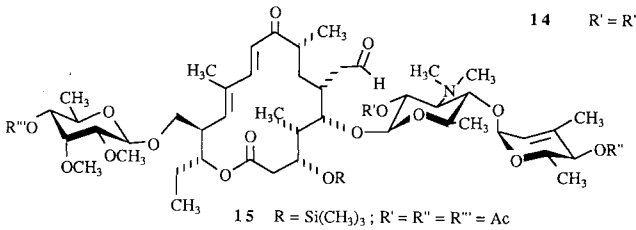


1 R' = R'' = R''' = R = H

13 R' = R'' = R''' = Ac ; R = H

14 R' = R'' = R''' = Ac ; R = $Si(CH_3)_3$



15 R = $Si(CH_3)_3$; R' = R'' = R''' = Ac

16 R = R' = H ; R'' = R''' = Ac

17 R = R' = R'' = R''' = H

furnish α,β-epoxy sulfones on treatment with chloromethyl *p*-tolyl sulfone in the presence of potassium *t*-butoxide[3,4]. Such a reaction, from unprotected tylosin (**1**), using 1 equiv. of *t*-BuOK gave an isomeric mixture of α,β-epoxy sulfones (**6**) in 69% yield. Treatment of **6** with 1 equiv. of $NaBH_4$ afforded 20-homo relomycin (**7**) and pyridinium chlorochromate oxidation of the latter gave the desired 20-homo tylosin (**8**). The yield of the last two steps, 30% and 20% respectively, was unsatisfactory. However, no improvement was attempted in view of the poor antibacterial properties of 20-homo tylosin (**8**). Structural proof for the latter was derived from its mass spectrum, indicating the incorporation of one more CH_2 group relative to tylosin and a general increase by 14 units in the fragmentation pattern of the parent macrolide involving its C-6 side-chain. Also, as expected, C-19 and C-20 moved to high and low field respectively with respect to these signals in the ^{13}C NMR spectrum of **1**[5].

In order to prepare 20-nor tylosin (**12**), a promising intermediate appeared to be 6-vinyl tylosin (**10**) (20-deoxy-19,20-didehydro tylosin). The latter was available by *m*-chloroperbenzoic acid-mediated elimination reaction of selenide **9**[6] with an overall yield of 45% from relomycin (**2**). However, various attempts, aiming at transforming **10** into 20-nor tylosin **12**, *via* 19 (*R* or *S*)-hydroxy relomycin, using $OsO_4/NaIO_4$ or $OsO_4/NaClO_3$ followed by $Pb(OAc)_4$ treatment, were found unsuccessful. 20-Nor tylosin (**12**) could eventually be prepared by a different approach, based on the photooxidation of an intermediate enamine[7]. Pyrrolidine reacted with tylosin (**1**) in the presence of molecular sieves giving in quantitative yield 20-deoxo-20-[(pyrrolidino)-imino] tylosin (**11**). Photooxygenation of the latter, in the presence of rose bengal afforded the desired 20-nor tylosin (**12**). Structural proof for **12** was derived from its mass spectrum indicating one less CH_2 group relative to tylosin and a general loss of 14 units in the fragmentation pattern of the parent macrolide (**1**) involving its C-6 side-chain. Furthermore, the ^{13}C NMR spectrum of 20-nor tylosin (**12**) exhibited, as expected, upfield shifts for C-5 and C-7 (79.5 and 28.2) and a strong downfield shift for C-6 (one of the five signals between 39~43 ppm) relative to the spectrum of tylosin (**1**) (C-5, C-6, C-7: 81.6; 32.9 and 32.2 ppm, respectively). The chemical shift of the aldehyde carbon of tylosin (**1**), 20-homo tylosin (**8**) and 20-nor tylosin (**12**) was almost identical (close to 203.0 ppm).

Another derivative of tylosin (**1**), namely 2″,3″-dehydro-3″-deoxy tylosin (**17**), considered to be a useful

intermediate for further transformations of the parent macrolide, was also prepared. When 2',4'',4'''-tri-*O*-acetyl-3-*O*-trimethylsilyl tylosin (**14**)[8,9] was treated, at low temperature, with thionyl chloride, regiospecific elimination of the mycarose tertiary hydroxyl group occurred. Liberation of the four hydroxyl groups could be achieved by sequential methanol and guanidine[10] treatment affording **17** with a satisfactory overall yield.

The *in vitro* antibacterial activity of four new compounds, **8**, **10**, **12** and **17**, was evaluated using *Staphylococcus aureus*, *Enterococcus* and *Haemophilus influenzae* organisms. 20-Homo tylosin (**8**) exhibited MIC values which were about twice higher than those for tylosin, the three other compounds showed only very much weaker antibacterial activity.

Experimental

Physico-chemical Determinations, Chromatography and Extraction

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker 200 MHz spectrometer. Thin layer chromatography (TLC) was performed using E. Merck plates of silica gel 60 with fluorescent indicator in CH_2Cl_2-CH_3OH-NH_4OH (90:9:1.5). Visualization was effected by spraying plates with 5% H_2SO_4 in ethanol followed by heating at 120~140°.

The term "usual work-up" means CH_2Cl_2 extraction, followed by washing with a diluted $NaHCO_3$ water solution, drying of the organic phase over Na_2SO_4 and evaporation under reduced pressure.

In Vitro Evaluation

These data were obtained by the standard microdilution methodology.

20-*C*-Cyano-20-deoxy-relomycin (**5**)

To a solution of **3** (1.25 g, 1.17 mmol) in acetone (20 ml) were added acetic anhydride (500 μl) and the mixture was stirred overnight at room temperature. After concentration and the usual work up of the residue, 99% pure 2'-*O*-acetyl-20-*O*-tosyl-relomycin (**4**) was obtained. To this product (1.01 g, 0.91 mmol) in acetonitrile (75 ml) was added KCN (0.11 g, 1.7 mmol) and dicyclohexano-18-crown-6 ether (0.72 g, 1.9 mmol) and the solution was stirred by sonication at 60°C for 11 hours and then concentrated. After the usual work-up and chromatography of the residue, starting material (0.26 g) and desired 2'-*O*-acetyl-20-*C*-cyano-20-deoxy-relomycin (0.30 g, 44%) were obtained. The latter was dissolved in methanol (20 ml) and the solution stirred at 40°C for 48 hours. After concentration and the usual work-up, 99% pure 20-C-cyano-20-deoxy-relomycin (**5**) was isolated, $[\alpha]_D$ −62° (*c* 0.75, $CHCl_3$), mass spectrum: *m/z* 927 (MH^+), ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 119.0 (CN). *Anal* Calcd for $C_{47}H_{78}N_2O_{16}$: C 60.91, H 8.42, N 3.02. Found: C 60.87, H 8.41, N 3.02..

Epoxy-sulfones (**6**)

To a solution of tylosin (**1**) (13.7 g, 14.7 mmol) in a solvent mixture of THF-*t*-BuOH 12:1 (100 ml) was added first *t*-BuOK (1.65 g, 14.7 mmol, 1 equiv.) and then slowly at 10°C chloromethyl *p*-tolylsulfone (3.07 g, 15 mmol). The mixture was stirred for 2 hours at 15°C then concentrated. After the usual work-up and chromatography, an isomeric mixture of epoxy-sulfones (**6**) (11 g, 69%) was obtained, mass spectrum: *m/z* 1084 (MH^+), ^1H NMR (200 MHz, $CDCl_3$) δ 7.80, 7.40, 2.50 (7H, tosylate).

20-Homo-tylosin (**8**)

To a solution of **6** (110 mg, 0.09 mmol) in DMF (5 ml) was added $NaBH_4$ (4 mg, 0.10 mmol). The mixture was stirred at 80°C for 75 minutes and then concentrated. Chromatography of the residue furnished 20-homo-relomycin (**7**) (25.7 mg, 30%), mass spectrum: *m/z* 932 (MH^+). To a solution of dichloromethane (8 ml) containing molecular sieves (4 Å, 60 mg) was added pyridinium chlorochromate (168 mg, 0.80 mmol) and stirring was maintained for 10 minutes. Then **7** (60 mg, 0.06 mmol) was added at room temperature in 2 hours. After the usual work-up 20-homo-tylosin (**8**) (11.9 mg, 20%) was obtained; $[\alpha]_D$ −68° (*c* 1.0, $CHCl_3$), mass spectrum: *m/z* 930 (MH^+), ^1H NMR (200 MHz, $CDCl_3$) δ 9.00 (s, 1H, aldehyde), ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 202.8 (C-24), 42.3 (C-20), 21.3 (C-19). *Anal* Calcd for $C_{47}H_{79}NO_{17}$: C 60.71, H 8.50, N 1.51. Found: C 60.82, H 8.37, N 1.58.

20-Deoxy-20-dihydro-20-(*o*-nitrophenyl)selenide Tylosin (**9**)

To a solution of relomycin (**2**) (20 g, 21.8 mmol) in THF (500 ml) was added *o*-nitrophenyl selenocyanide (20 g, 21.8 mmol) and then very slowly PBu_3 (16.3 ml, 65 mmol). Stirring was applied for 2 hours at room tempeature. After concentration and chromatography of the residue, **9** (10.43 g, 59%) was obtained, $[\alpha]_D$ −87° (*c* 1.0, $CHCl_3$), mass spectrum: *m/z* 1103 (MH^+). *Anal* Calcd for $C_{52}H_{82}N_2O_{18}Se$: C 56.67, H 7.45, N 2.54, Se 7.26. Found: C 56.85, H 7.37, N 2.44, Se 7.30.

6-Vinyl-tylosin (20-Deoxy-19,20-didehydro-tylosin) (**10**)

To a solution of **9** (10 g, 9.1 mmol) in dichloromethane (500 ml) was added *m*-chloroperbenzoic acid (3.13 g, 18 mmol) and the mixture was stirred for 75 minutes at room temperature. After concentration and chromatography of the residue, **10** (5.95 g, 73%) was obtained, $[\alpha]_D$ −51° (*c* 1.1, $CHCl_3$), mass spectrum: *m/z* 900 (MH^+), ^1H NMR (400 MHz, $CDCl_3$) δ 5.92 (m, 1H, H-19), 5.02 (m, 2H, H-20); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 140.1 (C-19), 115.9 (C-20). *Anal* Calcd for $C_{46}H_{77}NO_{16}$: C 61.40, H 8.56, N 1.56. Found: C 61.36,

H 8.70, N 1.55.

20-Nor-tylosin (**12**)

To a solution of tylosin (**1**) (550.2 mg, 0.6 mmol) in toluene (1.5 ml) was added molecular sieves (4 Å, 361 mg) and pyrrolidine (0.68 ml, 8.1 mmol) and the mixture was stirred at room temperature for 17 hours and then filtered. Concentration of the solution gave enamine **11** (557 mg, 99%), mass spectrum: *m/z* 969 (MH^+). To a solution of **11** (557 mg, 0.5 mmol) in a quartz vessel in DMF (30 ml) was added a catalytic amount of rose bengal (3.7 mg). Then, while oxygen gas was continuously passed through the solution, the latter was cooled to +15°C and irradiated by means of a neon source (2 × 15 W) for 4 hours. After concentration of the solution, the residue was purified by chromatography affording **12** (225 mg, 50%); $[\alpha]_D$ −70° (*c* 1.0, $CHCl_3$), mass spectrum: *m/z* 902 (MH^+), ^1H NMR (400 MHz, $CDCl_3$) δ 9.50 (s, 1H, H-19); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 203.3 (C-19). *Anal* Calcd for $C_{45}H_{75}NO_{17}$: C 59.93, H 8.32, N 1.55. Found: C 60.07, H 8.28, N 1.52.

2″,3″-Didehydro-3″-deoxy-tylosin (**17**)

To a solution of 2′,4″,4‴-tri-*O*-acetyl-tylosin (**13**) (600 mg, 0.57 mmol) in dichloromethane (6 ml) were added at 0°C pyridine (0.7 ml) and then slowly trimethylsilyl chloride (0.73 ml, 5.8 mmol). The mixture was stirred at 0°C for 1 hour and at room temperature for 16 hours. After dilution with water and the usual work-up a residue of crude, unstable, 2′,4″,4‴-tri-*O*-acetyl-3-*O*-trimethylsilyl-tylosin (**14**) (670 mg, 92%) was obtained. Without purification the latter was dissolved in pyridine (10 ml) and cooled to −40°C. To this solution was added in 2 hours a solution (8.4 ml) prepared from pyridine (4.1 ml), dichloromethane (4.1 ml) and thionyl chloride (263 µl). The mixture was stirred at −40°C for 1 hour and at 0°C for 5 hours. After the usual work-up and chromatography, **15** (503 mg, 80%) was obtained; mass spectrum: *m/z* 1096 (MH^+). Compound **15** (503 mg, 0.46 mmol) was refluxed for 3 hours in methanol (50 ml). After concentration of the solution, 4″,4‴-di-*O*-acetyl-2″,3″-didehydro-3″-deoxy-tylosin (**16**) (474 mg) was obtained. The latter was dissolved in anhydrous methanol (5 ml) and to this solution was added guanidine (30 mg, 0.5 mmol) and the mixture was stirred at room temperature for 45 minutes. After dilution with water, the usual work-up and chromatography, pure **17** (176 mg, 43%) was obtained; $[\alpha]_D$ −58° (*c* 1.0, $CHCl_3$), mass spectrum: *m/z* 898 (MH^+); ^{13}C NMR (50.33 MHz, $CDCl_3$) δ 141.0 (C-3″), 121.2 (C-2″), 18.0 (C-6″ and C-7″). *Anal* Calcd for $C_{46}H_{75}NO_{16}$: C 61.54, H 8.36, N 1.56. Found: C 61.74, H 8.25, N 1.49.

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