Studies on Some Derivatives of Oxamniquine

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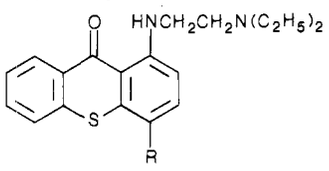
On the basis of the remarkable biological similarities between hycanthone and oxamniquine and as a sequel to our finding that some esters of hycanthone are active against hycanthone-resistant schistosomes, we prepared oxamniquine acetate, oxamniquine *N*-methylcarbamate, and four substituted phenylsulfonohydrazones of oxamniquine aldehyde. These compounds were tested for their effect on survival of and on [^{3}H]uridine incorporation into hycanthone-sensitive and -resistant *Schistosoma mansoni*. All of these derivatives were effective to a greater or lesser degree in killing worms and in inhibiting [^{3}H]uridine incorporation in the sensitive strain, but none was effective in the resistant strain.

Oxamniquine (OXA, **1**) was obtained originally as a fermentation product of UK-3883, **2**, with the aid of the same organism that had been used earlier to convert lucanthone (LC, **3**) to hycanthone (HC, **4**).[1] Although



3. R = CH_3 (lucanthone)
4. R = CH_2OH (hycanthone)

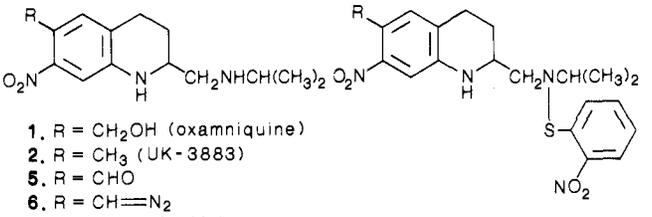
UK-3883 and LC are active against murine *Schistosoma mansoni* infections, they are inactive in vitro, whereas OXA (**1**) and HC (**4**) are active both in vivo and in vitro.[2] There is complete cross-resistance between HC and OXA in strains of *S. mansoni*, which are resistant to either drug. Because of these and other similarities, a mode of action for OXA was postulated that is very similar to that suggested earlier for HC.[1,3] In this mechanism OXA is enzymically esterified to give a reactive ester that acts as an alkylating agent and binds covalently to schistosomal DNA. As in the case of HC, resistance to OXA was attributed to the absence of the esterifying enzyme in the resistant strain. Such an interpretation of resistance was supported by the finding that some preformed esters of HC were active against HC-resistant schistosomes.[3,4] This prompted us to prepare some esters of OXA and test their activity against HC/OXA-sensitive and HC/OXA-resistant schistosomes.

In our hands attempts to prepare either sulfate or phosphate esters of OXA were unsuccessful. We succeeded in synthesizing the acetate and *N*-methylcarbamate esters as well as four arylsulfonohydrazones of oxamniquine aldehyde. The effect of these compounds on the survival of HC-sensitive and HC-resistant strains of *S. mansoni* was determined by using the methodology described previously.[3,4] In addition, the effect on schistosome [^{3}H]uridine incorporation was also determined, since it has been shown that there is a good correlation between the schistosomicidal activity of compounds belonging to the HC/OXA family and early irreversible inhibition of schistosome uridine incorporation.[2]

Chemistry

Since OXA contains two secondary amine groups, at least one may require protection during the acetylation of the alcohol function.

Oxidation of OXA with MnO_2 in CH_2Cl_2 gave the aldehyde **5** in a greater state of purity than reported previously.[5] Treatment of **5** with *o*-nitrobenzenesulfenyl



1. R = CH_2OH (oxamniquine)
2. R = CH_3 (UK-3883)
5. R = CHO
6. R = CH=N_2
11. R = $CH_2O_2CNHCH_3$
12. R = $CH_2O_2CCH_3$

7. R = CHO
8. R = CH_2OH
9. R = $CH_2O_2CNHCH_3$
10. R = $CH_2O_2CCH_3$

chloride gave the monosubstituted derivative **7**. Extensive NMR decoupling experiments showed that substitution occurred on the aliphatic rather than on the aromatic nitrogen. Reduction of **7** with $NaBH_4$ gave **8**, which was also prepared, albeit in lower yield, by treatment of **1** with *o*-nitrobenzenesulfenyl chloride. In this reaction substantial quantities of the O,N-disubstituted derivative were obtained. Acylation of **8** with CH_3NCO and acetic anhydride gave the esters **9** and **10**, respectively. Brief treatment with ethereal HCl furnished and required target compounds **11** and **12**.

Sartorelli and his co-workers reported that certain arylsulfonohydrazones of pyridine-2-carboxaldehyde *N*-oxide were antitumor agents by virtue of the fact that these compounds decomposed in vivo to give 2-pyridyldiazomethane *N*-oxide, which acted as an alkylating agent.[6] We prepared four phenylsulfonohydrazones (**13–16**) of oxamniquine aldehyde (**5**) in the hope that in the schistosome these hydrazones would decompose to the diazoalkane **6**, which could act as an alkylating agent.

Biological Results

The effect of oxamniquine *N*-methylcarbamate **11**, the corresponding acetate **12**, and the sulfonhydrazones **13–16** on [^{3}H]uridine incorporation by *S. mansoni* and worm survival in vitro are reported in Table I. To a greater or lesser degree, all compounds inhibited [^{3}H]uridine incorporation and caused death in the HC-sensitive strain, but none of the drugs was effective in the HC-resistant strain. In contrast, hycanthone *N*-methylcarbamate and hycanthone acetate were active in inhibiting [^{3}H]uridine incorporation and causing death in both strains.[3,4] We were surprised to find that the sulfonohydrazones were active

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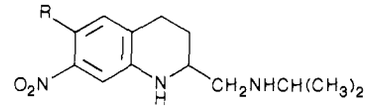
Table I. Properties of Oxamniquine Esters and Substituted Phenylsulfonohydrazones of Oxamniquine Aldehyde



no.	R	mp, °C	empirical formula	concn, μg/mL	% [^{3}H]uridine incorporation relative to controls (% worms surviving in culture)[a] HC-sensitive	HC-resistant
1	CH_2OH			50	14 (0)	97
				100	21 (0)	100
11	$CH_3NHCO_2CH_2$	125–127	$C_{16}H_{24}N_4O_4$	50	22 (ND)	100
				100	21 (ND)	100
12	$CH_3CO_2CH_2$	75–78	$C_{16}H_{23}N_3O_4$	50	10 (ND)	100
				100	20 (ND)	100
13	2,4,6-$(CH_3)_3C_6H_2SO_2NHN{=}CH$	189–190	$C_{25}H_{33}N_5O_6S$[b]	50	24 (0)	100
				100	32 (0)	100
14	4-$CH_3C_6H_4SO_2NHN{=}CH$	124–125	$C_{21}H_{27}N_5O_4S{\cdot}H_2O$[c]	50	60 (0)	102
				100	58 (0)	85
15	4-$NO_2C_6H_4SO_2NHN{=}CH$	190–192	$C_{22}H_{26}N_6O_8S$[b]	75	49 (0)	96
				150	32 (0)	104
16	4-$CH_3OC_6H_4SO_2NHN{=}CH$	193–200	$C_{23}H_{29}N_5O_7S$[b]	75	53 (0)	106
				150	25 (0)	100

[a] All treated HC-resistant schistosomes showed 100% survival. [b] Hemifumarate. Treatment of the arylsulfonohydrazones with aqueous base resulted in the formation of **6** as evidenced by the appearance of the characteristic absorption of the diazo groups at 2050 cm^{-1} in the IR spectrum. [c] Anal. Calcd for $C_{21}H_{27}N_5O_4S{\cdot}H_2O$: C, 54.40; H, 6.30; N, 15.11. Found: C, 54.60; H, 6.72; N, 14.46.

in the HC-sensitive strain but not in the resistant one. If these compounds behaved as those reported by the Sartorelli group,[6] then no enzymic activation is necessary for alkylation of the DNA from either the HC-sensitive or HC-resistant strain. The reason for the lack of activity of either the oxamniquine esters or the sulfonohydrazones in the HC-resistant strain is obscure.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are corrected. Infrared spectra were run on a Perkin-Elmer Model 298 infrared spectrometer. The proton NMR spectra were run on a 60-MHz Hitachi Perkin-Elmer R-600 spectrometer in $CDCl_3$ with $(CH_3)_4Si$ as the internal standard. Decoupling experiments were run on a Varian XL-200 spectrometer. Microanalyses were performed by Spang Microanalytical Laboratory, Eagle Harbor, MI, and Atlantic Microlab, Inc., Atlanta, GA, and were within ±0.4% of the theoretical values unless stated otherwise.

Oxamniquine Aldehyde (5). One gram (3.6 mmol) of oxamniquine was dissolved in 100 mL of CH_2Cl_2, and to the stirred solution was added 3 g of MnO_2 in two portions in 15 min. The mixture was stirred overnight and filtered, and the residue left after removal of the solvent was chromatographed on silica with $CHCl_3$ as the eluant. The aldehyde melted at 99–100 °C (lit.[5] mp 93–94 °C) after crystallization from CH_2Cl_2–hexane (wt 880 mg, 89%). Anal. ($C_{14}H_{19}N_3O_3$) C, H, N.

Reduction of 50 mg of this aldehyde in 15 mL of CH_3OH with 50 mg of $NaBH_4$ at room temperature until complete (TLC) gave 45 mg of oxamniquine.

N^2-[(2-Nitrophenyl)thio]oxamniquine Aldehyde (7). A solution of 600 mg (2.16 mmol) of oxamniquine aldehyde, 0.5 mL of triethylamine, and 600 mg (3.16 mmol) of 2-nitrobenzenesulfenyl chloride in 10 mL of CH_2Cl_2 was stirred at room temperature for 1 h. The solvent was removed, leaving a residue, which, after chromatography on silica with $CHCl_3$ as the eluant followed by crystallization from CH_2Cl_2–hexane, gave 830 mg (90%) of the product, mp 180–181 °C. Anal. Calcd for $C_{20}H_{22}N_4O_5S$: C, 55.80; H, 5.15; N, 13.02. Found: C, 55.74; H, 5.37; N, 12.56.

N^2-[(2-Nitrophenyl)thio]oxamniquine (8). The above aldehyde (200 mg, 0.46 mmol) was dissolved in 50 mL of CH_2Cl_2–CH_3OH (1/1) and treated with 400 mg of $NaBH_4$. After 2 h of being stirred at room temperature, the mixture was evaporated to dryness in vacuo, and the residue was dissolved in CH_2Cl_2. Evaporation of the solvent left 190 mg (95%) of almost pure alcohol suitable for use in the next stage. After crystallization from CH_2Cl_2–hexane, it melted at 155 °C. Anal. ($C_{20}H_{24}N_4O_5S{\cdot}0.5H_2O$) C, H, N: calcd, 12.55; found, 12.59.

N^2-[(2-Nitrophenyl)thio]oxamniquine N-Methylcarbamate (9). A solution of 220 mg (0.51 mmol) of **8**, 0.5 mL (0.84 mmol) of methyl isocyanate, and 100 mg (0.87 mmol of 4-(dimethylamino)pyridine in 25 mL of CH_2Cl_2 was stirred at room temperature for 12 h. Evaporation of the solvent followed by chromatography of the residue on silica ($CHCl_3$) furnished 245 mg (98%) of the carbamate, which, after crystallization from CH_2Cl_2–hexane, melted at 138 °C (wt 200 mg, 80%). Anal. ($C_{22}H_{27}N_5O_6S$) C, H, N.

Oxamniquine N-Methylcarbamate (11). The above NPS derivative (100 mg, 0.20 mmol) was dissolved in 100 mL of dry ether, and to this solution was added dropwise 2 mL of 3 N ethereal HCl. The hydrochloride that separated was collected, washed thoroughly with ether, and then dissolved in 20 mL of H_2O. A solution of $NaHCO_3$ (200 mg in 5 mL of H_2O) was added, and the aqueous phase was extracted with 2 × 25 mL of CH_2Cl_2. The combined extracts were concentrated, and the residue was chromatographed on a silica gel plate with $CHCl_3$–acetone–$(Et)_3N$ (70:30:5) as the developing solvent. There was obtained 40 mg of the desired carbamate, after crystallization from CH_2Cl_2–hexane: NMR δ 1.15 (d, 6 H, 2 CH_3), 2.80 (t, 2 H, CH_2), 2.90 (s, 3 H, $NHCH_3$), 2.95 (d, 2 H, CH_2), 3.40 (m, 2 H, CH_2), 3.55 (s, 1 H, NH), 5.35 (s, 2 H, CH_2O), 7.20–7.30 (s, 2 H, aromatic H). Anal. ($C_{16}H_{24}N_4O_4$) C, H, N.

Oxamniquine Acetate (12). A solution of 100 mg (0.25 mmol) of **8**, 0.05 mL of acetic anhydride, and 50 mg (0.41 mmol) of 4-(dimethylamino)pyridine in 25 mL of CH_2Cl_2 was stirred at room temperature for 3 h. The solution was evaporated, and the residue was chromatographed on silica ($CHCl_3$) to give 93 mg (80%) of the crude acetate: mp 70–75 °C; NMR δ 1.25 (d, 2 H, CH_3), 2.0 (s, 3 H, $COCH_3$), 2.70–3.50 (m, 7 H), 5.30 (s, 2 H, CH_2), 7.10 (s, 1 H), 7.50–8.40 (m, 6 H, arom).

A solution of 60 mg of the above acetate in 20 mL of anhydrous ether was treated with 1.0 mL of 3 N ethereal HCl. The hydrochloride that separated was filtered, dissolved in H_2O, and treated with $NaHCO_3$ solution. The suspension was extracted with 2 × 25 mL of CH_2Cl_2. The organic phase was concentrated to dryness to leave a residue, which, after chromatography on silica gel and crystallization from CH_2Cl_2–hexane, weighed 25 mg (62%): NMR δ 1.20 (d, 6 H, 2 CH_3), 2.1 (s, 3 H, $OCCH_3$), 2.80–3.43 (8 H, CH_2CH, NH), 5.32 (s, 2 H, CH_2), 7.20–7.30 (2 s, 2 H, arom). Anal. ($C_{16}H_{23}N_3O_4$) C, H; N: calcd, 13.18; found; 12.70.

Preparation of Substituted Phenylsulfonohydrazones of Oxamniquine Aldehyde as Fumarate Salts. To a solution of 554 mg (2.0 mmol) of oxamniquine aldehyde in 25 mL of CH_3OH there was added 235 mg (2.02 mmol) of fumaric acid dissolved

in 10 mL of CH_3OH. After 2 h at room temperature, the suspension was filtered to give 600 mg of the desired salt, mp 203–204 °C.

A solution of 1.5 g (0.4 mmol) of the above fumarate in 14 mL of CH_3OH and 2 mL of H_2O was treated with 0.45 mmol of the requisite phenylsulfonohydrazine in 6 mL of CH_3OH. The mixture was allowed to stand overnight and filtered. The salts were purified by crystallization from aqueous ethanol.

The *p*-tolyl derivative was converted to the free base and crystallized from CH_3OH. The overall yield of the free base was 66% (Table I).

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Registry No. **1**, 21738-42-1; **5**, 53503-66-5; **5**·$^1/_2C_4H_4O_4$, 114719-39-0; **7**, 114719-26-5; **8**, 114719-27-6; **8** (acetate), 114719-30-1; **9**, 114719-28-7; **11**, 114719-29-8; **12**, 114719-31-2; **13**, 114719-32-3; **13**·$^1/_2C_4H_4O_4$, 114719-33-4; **14**, 114719-34-5; **15**, 114719-35-6; **15**·$^1/_2C_4H_4O_4$, 114719-36-7; **16**, 114719-37-8; **16**·$^1/_2C_4H_4O_4$, 114719-38-9; 3-nitrobenzenesulfenyl chloride, 7669-54-7; [(2,4,6-trimethylphenyl)sulfonyl]hydrazine, 16182-15-3; (4-tolylsulfonyl)hydrazine, 1576-35-8; [(4-nitrophenyl)sulfonyl]-hydrazine, 2937-05-5; [(4-methoxyphenyl)sulfonyl]hydrazine, 1950-68-1.

Synthesis and Evaluation of Tylosin-Related Macrolides Modified at the Aldehyde Function: A New Series of Orally Effective Antibiotics

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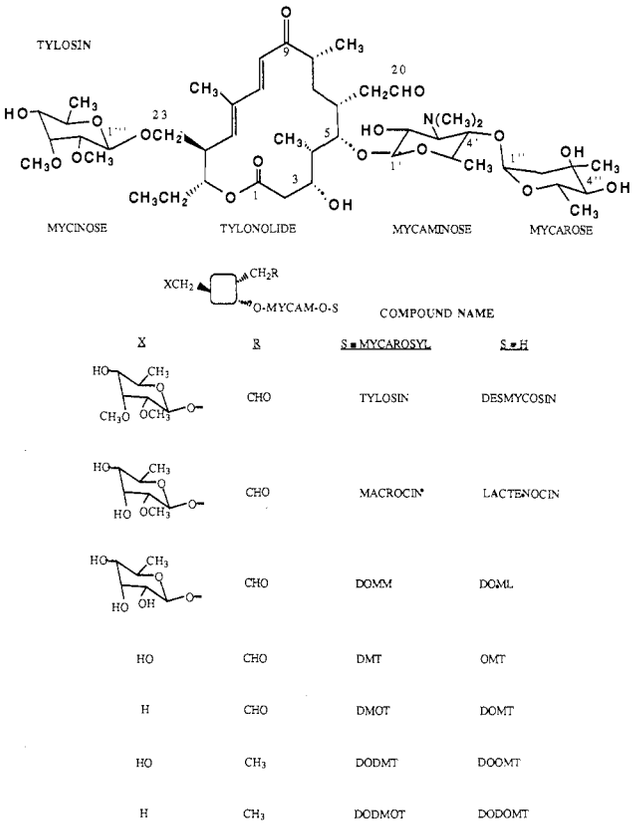
Modification of the aldehyde group in tylosin and related macrolide antibiotics dramatically enhanced the oral efficacy of the derivatives against experimental infections caused by susceptible bacteria in laboratory animals. A large number and wide variety of aldehyde-modified macrolide derivatives were prepared, utilizing the Mitsunobu reaction and other chemical transformations. Evaluation of in vitro and in vivo antimicrobial activity indicated that derivatives of demycarosyltylosin (desmycosin) combined the broadest spectrum of antimicrobial activity with the best efficacy and bioavailability after oral administration.

Biosynthetically blocked mutant strains of the tylosin-producing microorganism *Streptomyces fradiae* have now made available a wide variety of biosynthetic intermediates and shunt metabolites in quantities sufficient for extensive chemical modification studies (Scheme I).[1-4] In this paper, we report the results from one line of investigation that has yielded a new series of semisynthetic antibiotics having increased oral efficacy and bioavailability in laboratory animals.

Initial evaluation of the antimicrobial activity of these tylosin-related biosynthetic intermediates and shunt metabolites suggested that the ratio of oral/subcutaneous efficacy against model experimental infections in mice was substantially lowered for 20-deoxo-5-*O*-mycaminosyltylonolide (DOOMT) compared to 5-*O*-mycaminosyltylonolide (OMT).[5] Prior to our study, the only modification of the aldehyde of tylosin had been reduction to its dihydro derivative, relomycin.[6] Consequently, modification of the aldehyde group was more extensively investigated in both tylosin and this newly available collection of 16-membered macrolides.

Initial Survey. In order to prepare a group of analogues of DOOMT, deformylation of the aldehyde function was investigated for tylosin and related macrolides by utilizing Wilkinson's catalyst [$(Ph_3P)_3RhCl$]. Tylosin, macrocin, desmycosin, 23-demycinosyltylosin (DMT), and OMT were all readily converted to their deformyl derivatives (Scheme II). After our work had been completed, workers in another laboratory independently reported similar results from decarbonylation reactions.[7] A group

Scheme I. Tylosin-Related Macrolides Available as Starting Materials for Chemical Modification



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