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CHEMICAL CONVERSION OF MOCIMYCIN TO AURODOX AND DERIVATIVES OF AURODOX, GOLDINAMINE AND MOCIMYCIN

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Mocimycin was converted to the acylesters by selective acylation of the hydroxyl group of the 4-hydroxy-1-methyl-2(1H)pyridinone moiety. Subsequent N-methylation at the nuclear nitrogen and removal of the protective group from the resulting reaction products afforded aurodox. Mono-O-acetylmocimycin and several analogous aurodox esters thus prepared possess antibacterial activity in vitro and growth-promotion properties in poultry.

Esters of aurodox involving the hydroxyl group of the 4-hydroxy-1-methyl-2(1H)pyridinone moiety are activated. Accordingly, acetic acid treatment of the aurodox esters generates O-acylgoldinamines which undergo transacylation furnishing N-acylgoldinamines. Alternatively, N-acylgoldinamines can be prepared by selective mono-O-arylsulfonylation of aurodox, liberating O-arylsulfonylgoldinamine by treatment with acetic acid followed by N-acylation and removal of the protective arylsulfonyl group.

A third approach to N-acylgoldinamines consists in direct N-acylation of goldinamine itself which is prepared by acetic acid treatment of aurodox. None of these derivatives prepared, however, exhibited significant antimicrobial or growth-promoting properties, suggesting that the goldinonic acid moiety, or a closely related derivative thereof, is required for biological activity.

Mocimycin (1) and aurodox (2)[1] are members of a new class of antibiotics for which the cumulative term elfamycins* is proposed. These two antibiotics differ only by an N-methyl group which is absent in mocimycin. Both 1 and 2 possess limited antimicrobial in vivo spectra but exhibit pronounced activities as growth promoters in poultry. Because aurodox demonstrated superior performance in this respect, a conversion of mocimycin to aurodox became of interest.

This conversion was first attempted with cell-free extracts of the aurodox-producing Streptomyces goldiniensis.[2] Lack of success, however, prompted the search for a chemical alternative.

Treatment of 1 sodium salt with methyl iodide yielded a mixture of mono- and dimethylated products, 1a and 2a, respectively; formation of 2a proceeded with initial O-methylation followed by N-methylation.[3] To effect specific N-methylation of 1, resulting in a conversion of 1 to 2, it was necessary to protect the 4-hydroxy function of the pyridone moiety of mocimycin prior to the methylation step. We investigated acyl- and arylsulfonyl-groups for this purpose and found the acyl groups removable under the mildest conditions, ranging from treatment with aqueous pyridine to short exposure to aqueous ammonium hydroxide at room temperature.

Mocimycin sodium salt in dimethylformamide or mocimycin free-acid in pyridine reacted with phenylglyoxyloyl chloride[4,5] to afford ester 1b, and mocimycin sodium salt in dimethylformamide react-

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* The term elfamycin was chosen in view of the specific ability of these antibiotics to inhibit protein synthesis by interaction with elongation factors.
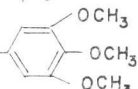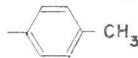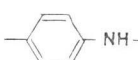
ed readily with acetic anhydride to yield ester **1c**; both esters could be purified by gel permeation chromatography. Methylation of **1b** and **1c** was effected with methyl iodide and silver oxide in dimethyl formamide within two hours yielding the *N*-methylated esters **2b** and **2c**, respectively, isolated after purification by gel permeation chromatography.

To prove the structures of **2b** and **2c** derived from mocimycin, aurodox was reacted with phenylglyoxyloyl chloride and acetic anhydride independently to yield compounds identical with **2b** and **2c** derived from mocimycin.

The protective phenylglyoxyloyl group of **2b** was removed with aqueous pyridine at room temperature and the protective acetyl group of **2c** was removed by ammoniacal methanol in minutes at room temperature both affording **2** which was purified either by preparative thin-layer chromatography or, for larger scale preparations, by gel permeation chromatography of the sodium salt.[6]

Aurodox prepared from mocimycin proved to be identical in all respects with authentic **2** including spectral properties, mobility on tlc and bioactivity.[6] The overall yield for the conversion of mocimycin (**1**) to aurodox (**2**) was $12 \sim 20\%$. No effort was made to optimize yields.
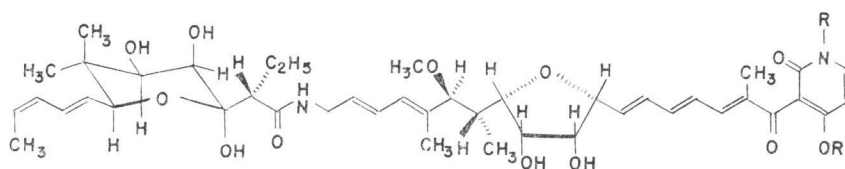
1 : R = R' = H (Mocimycin)

2 : R = Me, R' = H (Aurodox)

1a : R = H, R' = Me (Mocimycin methyl ether)

2a : R = R' = Me (Aurodox methyl ether)

1b : R = H, R' = CO-CO-C₆H₅

2b : R = Me, R' = CO-CO-C₆H₅

1c : R = H, R' = CO-CH₃

2c : R = Me, R' = CO-CH₃

2d : R = Me, R' = CO-C₆H₅

2e : R = Me, R' = CO— (trimethoxyphenyl: OCH₃, OCH₃, OCH₃)

2f : R = Me, R' = SO₂—C₆H₄—CH₃

2g : R = Me, R' = SO₂—C₆H₄—NH-Ac

It is noteworthy that acylated mocimycin and aurodox exhibited *in vitro* activity, thus prompting the preparation of various esters such as **2d**, **2e**, **2f** and **2g** all of which exhibited some degree of *in vitro* activity.[7] Interestingly, the antimicrobial spectra of these aurodox esters change considerably with the nature of the ester group and it appears that those aurodox esters whose ester linkage

would be expected to undergo hydrolysis under milder conditions turned out to possess superior antimicrobial spectra *in vitro*. Thus, **1c** and **2c** were comparable to **1** and **2** in *in vitro* activity against Gram-positive bacteria, whereas the benzoic and benzenesulfonic esters of **1** and **2** exhibited generally much lower activity, although *Bacillus megatherium* and *Streptomyces cellulosae* maintained their sensitivity to all aurodox esters to varying extents.

In spite of these differences in antibacterial activity, most esters of **1** and **2** were highly active in increasing growth rate and improving feed conversion at low dietary concentrations (Table 1). The performance data for **1c** and **2c** were comparable to those obtained with lincomycin at 2.2 mg/kg.

Although goldinamine ethers of type **4** played an important role in the structural elucidation of aurodox and mocimycin, goldinamine (**3**) itself has not been described previously. We have now succeeded in the isolation of **3** and prepared various *N*- and *O*-acyl derivatives thereof. Their synthesis was accomplished by three different methods. Treating *O*-acyl aurodox (**2c**~**2e**) with acetic acid cleaved the central amide bond and liberated the corresponding *O*-acylgoldinamine (**5a**~**5c**). In view of the activated-ester nature of *O*-acylgoldinamines transacylation occurred immediately yielding the desired *N*-acylgoldinamines **6a**~**6c** directly. The stability of arylsulfonic esters, however, is sufficient to preclude arylsulfonyl migration. In another approach, consequently, fission of *O*-arylsulfonylaurodox led to *O*-arylsulfonylgoldinamine (**5d** and **5e**) serving as a suitable starting material for goldinamine deriva-

Table 1. Growth-promotant activity of various compounds in chicken.

Compound	Concentration of compound in g/ton of feed	Weight gain, %	Feed conversion, %
Aurodox (**1**)	10	117	106
Lincomycin	4	120	108
Bambermycin	2	115	104
Virginia-mycin	5	108	104
1c	10	117	106
2c	10	111	102
2d	10	118	101
2e	10	115	104
2f	10	107	93
2g	10	114	100
3	10	101	106
6a	10	97	103
6b	10	105	92
6c	10	106	95
6d	10	101	102
Basal (control)	0	100	100

Average data for 3 replicates of 6 birds each (3 females and 3 males); the chickens were 3 days old at the time of testing; feeding period was two weeks (14 days).
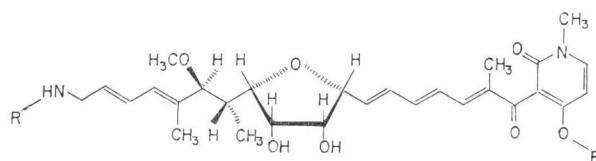
3 : R = R' = H (Goldinamine)

4 : R = H , R' = CH₂—⟨ ⟩—Br

5a : R = H, R' = COCH₃

5b : R = H, R' = COC₆H₅

5c : R = H, R' = CO—⟨ ⟩ (OCH₃)₃

5d : R = H, R' = SO₂—⟨ ⟩—CH₃

5e : R = H, R' = SO₂—⟨ ⟩—NH—Ac

6a : R = COCH₃, R' = H

6b : R = COC₆H₅, R' = H

6c : R = CO—⟨ ⟩ (OCH₃)₃, R' = H

6d : R = (N-t-BOC)glycyl , R' = H

tives as the protective arylsulfonyl groups are removable with ammonium hydroxide at room temperature. Finally, *N*-acylgoldinamine could be prepared by *N*-acylation of **3** itself.
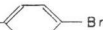
None of the *N*- or *O*-acylgoldinamines prepared showed significant antibiotic or growth-promotion properties in chickens suggesting the requirement of the goldinonic acid moiety, or a close relative thereof, to impart biological activity to goldinamine.

Experimental

Tlc was performed with precoated plates (silica gel 60 F-254, Merck, Darmstadt; preparative plates, silica gel P F-254, contained layers of 2-mm thickness) and were developed with system 1 (chloroform - methanol, 9: 1, v/v), 2 (chloroform - methanol - conc.ammonium hydroxide, 40: 10: 1, v/v), or 3 (chloroform - methanol, 8: 1, v/v). All concentrations were carried out under reduced pressure.

Mocimycin phenylglyoxylic ester (1b)

Procedure A. Mocimycin sodium salt (500 mg, 0.61 mmol) was dissolved in dimethylformamide (5.5 ml) and 0.7 ml of a solution containing approximately 0.7 mmol of phenylglyoxyloyl chloride, prepared by diluting phenylglyoxyloyl chloride (1 g, 5.93 mmol) with benzene (5 ml), was added. The mixture was kept in the dark overnight and was equilibrated with a mixture of water (10 ml) and chloroform (25 ml). The lower phase was washed consecutively with saturated sodium hydrogen carbonate solution (15 ml) and water (15 ml), concentrated to dryness, redissolved in acetone (2 ml) and chromatographed on a column (50 × 475 mm) of Sephadex LH-20 with acetone as mobile phase. The column effluent was collected in fractions of 10 ml each, and the composition of the fractions was monitored by tlc (system 1). Compound 1b was eluted as the major band (Rf 0.23) essentially free of minor impurities which were eluted immediately before (Rf 0.26, 0.30) and after (Rf 0.12, 0.16 [unreacted mocimycin], 0.18). Fractions containing pure 1b were pooled and concentrated to dryness to yield 182 mg of 1b (0.196 mmol, 32%) as amorphous, yellow powder with an NMR spectrum similar to that of mocimycin but with additional signals characteristic for the phenylglyoxyloyl group, $\delta_{TMS}^{CDCl_3}$ 3.17 (s, OCH_3), 7.47 (m, 3H of phenyl group overlapping with H-6 of pyridone moiety) and 7.95 (d, 2H of phenyl group, $J_0 = 8$ Hz).

Procedure B. Mocimycin (250 mg, 75% purity, 0.235 mmol) was dissolved in anhydrous pyridine (2.5 ml). The solution was cooled in an ice bath and 0.6 ml of the phenylglyoxyloyl chloride solution described in Procedure A was added under stirring. After 5 minutes the solution was equilibrated with a mixture of chloroform (25 ml), saturated sodium hydrogen carbonate solution (15ml) and crushed ice. The chloroform phase was washed twice with ice-cold water, concentrated to dryness, the residue was dissolved in acetone and chromatographed as described previously, to yield 115 mg of 1b (0.124 mmol, 53%).

Mocimycin acetic ester (1c)

Mocimycin sodium salt (2.10 g, 2.56 mmol) was dissolved in dimethylformamide (10 ml) and acetic anhydride (0.5 ml) was added. After stirring for 30 minutes, the reaction mixture was quenched by adding it to a stirred mixture of saturated sodium hydrogen carbonate solution (100 ml) and chloroform (75 ml). After 5 minutes of stirring, the resultant mixture was transferred to a separatory funnel containing chloroform (50 ml) and the phases equilibrated. Discarding the aqueous phase, the chloroform phase was washed again with saturated sodium hydrogen carbonate solution. The dried chloroform phase (sodium sulfate) was concentrated until most of the dimethylformamide was removed. The residue was dissolved in chloroform (*ca* 10 ml) and added to petroleum ether (*ca* 500 ml) to precipitate crude 1c (1.82 g, 2.17 mmol). The purity of this material depends greatly on the purity of the mocimycin used. Purification of 1c was achieved by chromatography on a column of Sephadex LH-20 with methanol. $\delta_{TMS}^{CDCl_3}$ 2.12 (s, CH_3CO).

Aurodox phenylglyoxylic ester (2b) from 1b

A mixture of 1b (182 mg, 0.196 mmol), silver oxide (approx. 100 mg), dimethylformamide (9 ml) and methyl iodide (0.37 ml) was shaken for 70 minutes at room temperature. The suspension was filtered and the filtrate was equilibrated with ethyl acetate (25 ml) and water (50 ml). The ethyl acetate phase was filtered, concentrated to a thin syrup, diluted with acetone (2 ml) and chromatographed on a column (25 × 450 mm) of Sephadex LH-20 with acetone as mobile phase. Fractions

containing 5 ml each were collected and analyzed by tlc (system 1), fractions containing the major band (Rf 0.30) were largely free of impurities which were eluted immediately before (Rf 0.33, 0.39, 0.46) and after (Rf 0.16, 0.20, 0.25) the major band. Concentration of the pooled fractions afforded 103 mg of **2b** as yellow, amorphous powder (0.109 mmol, 55.6%). The NMR spectrum of **2b** is very similar to that of **1b** with the exception of a signal for an *N*-methyl group, $\delta_{TMS}^{CDCl_3}$ 3.17 (s, OCH₃), 3.58 (s, N-CH₃), 7.39 (d, H-6 of pyridone moiety, J=8 Hz) overlapping with 7.46 (m, 3H of phenyl group) and 7.96 (d, 2H of phenyl group, J₀=8 Hz).

Aurodox acetic ester (2c) from (1c)

Crude **1c** (182 mg, 0.217 mmol) was methylated and purified as described previously for the preparation of **2b** to yield **2c** (48 mg, 0.056 mmol), $\delta_{TMS}^{CDCl_3}$ 2.12 (s, CH₃CO) and 3.53 (s, N-CH₃).

Aurodox phenylglyoxylic ester (2b) from 2

Aurodox sodium salt (500 mg, 0.60 mmol) was dissolved in dimethylformamide and the phenyl-glyoxyloyl chloride solution (2.5 ml) described previously was added. The reaction mixture was kept in the dark overnight and was equilibrated with ethyl acetate (100 ml) and water (75 ml). The ethyl acetate phase was washed consecutively with saturated sodium hydrogen carbonate solution (75 ml) and water (75 ml), dried with sodium sulfate, concentrated to a syrup and chromatographed as described for the preparation of **2b** from **1b**, to yield **2b** (131 mg, 23%).

Aurodox acetic ester (2c) from 2

Aurodox sodium salt (8.6 g, 10.3 mmol) was dissolved in dimethylformamide (100 ml) and acetic anhydride (2.2 ml) was added. After stirring for 30 minutes, the reaction mixture was quenched by adding it to a stirred mixture of saturated sodium hydrogen carbonate solution (250 ml) and chloroform (150 ml). After 20 minutes of stirring the resultant mixture was transferred to a separatory funnel containing chloroform (300 ml) and the phases were equilibrated. Discarding the aqueous phase, the chloroform phase was washed again with saturated sodium hydrogen carbonate solution followed by four water washes. The dried chloroform phase (sodium sulfate) was concentrated at 55°C to a very thick syrupy residue which was dissolved in chloroform (50 ml) and added to petroleum ether (1,200 ml) to precipitate crude **2c** (8.77 g, 10.3 mmol). Purification of **2c** was achieved by chromatography on a column of Sephadex LH-20 with acetone, Rf 0.48 (system 3), $\delta_{TMS}^{CDCl_3}$ 2.12 (s, CH₃CO).

Aurodox (2) from 2b

A solution of **2b** (103 mg, 0.109 mmol) in pyridine (2.5 ml) and water (2.5 ml) was kept at room temperature for 7 days, concentrated to a thin syrup and redissolved in chloroform. This solution was washed twice with saturated sodium hydrogen carbonate solution, once with water, concentrated and applied to two preparative tlc plates. The plates were developed with system 2; the major band (Rf 0.13) was eluted with chloroform - methanol, 4: 1. The aurodox ammonium salt (37 mg, 0.045 mmol, 41%) could be converted to the free acid by equilibrating with sodium dihydrogen phosphate solution and methylene chloride, followed by washing of the methylene chloride solution with water, drying and concentration to dryness.

Aurodox (2) from 2c

A solution of **2c** (24 mg, 0.028 mmol) in ammoniacal methanol (3 ml of a 30 mg NH₃/ml methanol solution) was kept at room temperature for 5 minutes. The solution was concentrated to dryness to yield **2** (20 mg, 0.025 mmol) after conversion to the free acid as described above.

Aurodox esters 2d, 2e, 2f and 2g (general procedure)

Aurodox sodium salt (2.2 g, 2.64 mmol) was dissolved in dimethylformamide (25 ml) and cooled in an ice bath. Acid chloride (3 mmol) was added and stirred vigorously for 30 minutes whereupon the reaction mixture was quenched by adding it to a stirred mixture of saturated sodium hydrogen carbonate (100 ml) and chloroform (25 ml). After 20 minutes of stirring the mixture was transferred to a separatory funnel containing chloroform (25 ml) and the phases were equilibrated. Discarding the aqueous phase, the chloroform phase was washed again with saturated sodium hydrogen carbonate solution followed by two water washes. The dried chloroform phase (sodium sulfate) was con-

centrated at 55°C to a very thick syrup which was dissolved in acetone and purified by chromatography on a column of Sephadex LH-20. The appropriate fractions were pooled and concentrated to solids.

N-Acylgoldinamines (6) via transacylation of O-acylgoldinamines (5)

The appropriate aurodox ester (2 mmol) was dissolved in acetic acid (30 ml), the solution was heated on the steam bath for 25 minutes and evaporated. The residue was redissolved in methanol and refluxed for 2 hours to effect the transacylation. The ninhydrin-negative product could be separated from goldinono-1,4-lactone-3,7-hemiacetal (Rf 0.30, system 2; Rf 0.65, system 3) by chromatography on Sephadex LH-20 with methanol as mobile phase.

Evaporation of the appropriate fraction yielded 5 as an amorphous, yellow powder in $60 \sim 70\%$ yield, normally exhibiting several spots on the tlc due to tautomeric equilibria. To obtain nearly homogeneous 5 the mixture (100 mg) was treated either by dissolving it in methanol - conc.ammonium hydroxide (3: 1, 4 ml) and allowing the solution to stand in the dark at room temperature up to 24 hours, or by dissolving it in water - pyridine (2: 1, 4 ml) and heating the solution on the steam bath for 4 hours. The residue obtained after evaporation was dissolved in chloroform, the solution washed with 0.5 M sodium dihydrogen phosphate and water, and evaporated. Chromatography on Sephadex LH-20 with acetone as mobile phase gave 5. Generally, more homogeneous materials were obtainable by immediately redissolving the chloroform concentrate in methanol, adjusting the pH to 9 with sodium methoxide and chromatographing the concentrated solution on Sephadex LH-20 as the sodium salt.

As an example, 6b was obtained as a substantially homogeneous substance after a 30-minute treatment with methanolic ammonium hydroxide prior to chromatography and work-up, Rf 0.70 (system 2), 0.22 (system 3), $\delta_{TMS}^{DMSO-d6}$ 0.76 (d, $\rangle$CH–CH$_3$, J=7 Hz), 1.61 (s, $\rangle$C–CH$_3$), 1.88 (s, =C(CH$_3$)–CO), 2.07 (m, –CH(CH$_3$)–), 3.07 (s, OCH$_3$), 3.32 (d, $\rangle$CHOCH$_3$, J=9 Hz), 3.33 (s, N–CH$_3$), 3.75 (m, $\rangle$CH–O), 3.85~4.25 (m, 5H, –CH$_2$N, 3 $\rangle$CH–O), 4.47 (broad, 2OH), 5.65~6.95 (8 –CH=), 5.98 and 7.59 (AB, J=7.5 Hz), 7.48 (m, 3H, arom.), 7.88 (m, 2H, arom.), and 8.65 (t, J=5.5 Hz, –CH$_2$NH--). The ^{1}H NMR spectrum of 6b sodium salt was very similar with the following diagnostic exceptions of general significance: 5.35 and 7.04 (AB, J=7.5 Hz), and 3.12 (s, N–CH$_3$).

N-Acylgoldinamines (6) via O-(p-toluenesulfonyl)-goldinamine (5d)

A solution of aurodox ester 2f (2 g) in acetic acid (50 ml) was heated on the steam bath for 25 minutes and evaporated. The residue was chromatographed on a column of Sephadex LH-20 with methanol as mobile phase. The fraction containing the ninhydrin-positive solute (Rf 0.75, system 2; Rf 0.06, system 3; golden yellow color) was evaporated to yield 5d acetate (0.92 g). N-Acylation was carried out in a conventional fashion with methanol, 80% aqueous ethanol or dimethylformamide as solvents. The product was isolated by extraction with chloroform, evaporation of the extract after washing with sodium hydrogen carbonate and water and chromatography on Sephadex LH-20 with methanol or acetone as mobile phase. To remove the protective group a solution of the N-acyl-O-(p-toluenesulfonyl)goldinamine in methanol - conc.ammonium hydroxide (3: 1), was allowed to stand overnight. Chromatography of the free acid or the sodium salt of 6 was carried out as described in the preceding example.

N-Acylgoldinamine (6) via acylation of 3

A solution of 1 (2.1 g, sodium salt) in acetic acid (50 ml) was heated on the steam bath for 25 minutes and concentrated. The oily residue was taken up in methanol - water (2: 1, 13 ml) further diluted with methanol (6 ml) and passed through a column of Dowex 50-X2 (Py$^+$, 50 ml, previously washed with methanol - water - pyridine, 6: 3: 1, 100 ml). The column was washed with the same aqueous methanol-pyridine mixture (100 ml) and then eluted with methanol - 5 M ammonium hydroxide (2: 1, 200 ml). The ammoniacal fractions were immediately concentrated, most of the water was azeotropically removed with propanol and the residue chromatographed on a column of Sephadex LH-20 (51 mm × 750 mm) with methanol as mobile phase. Evaporation of the appropriate fractions gave 3 ammonium salt as deep yellow powder (0.61 g) consisting of one major component (golden yellow color with ninhydrin, Rf 0.07, system 2), and two minor ninhydrin-

positive tautomers with higher Rf values. The crude goldinamine thus obtained was distributed between 0.5 M sodium dihydrogen phosphate solution and dichloromethane; the acylating agent was added immediately to the dichloromethane phase. The product was isolated as usual either as the free acid or the sodium salt by chromatography on Sephadex LH-20.

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