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THIAZO RIFAMYCINS II

MECHANISM OF THE REACTION BETWEEN RIFAMYCIN S AND 2-AMINO ETHANETHIOL DERIVATIVES

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Abstract—A mechanism for the reaction between rifamycin S and HS–CH_2–$CH(NH_2)$–R derivatives, leading to the formation of thiazole and thiazin-2-one rifamycin derivatives is proposed. It is shown that the ring-contraction thiazine thiazole occurs with extrusion of the CH_2 group to the S atom, while the formation of the thiazole and the thiazin-2-one rifamycins depends on the nature of the R group.

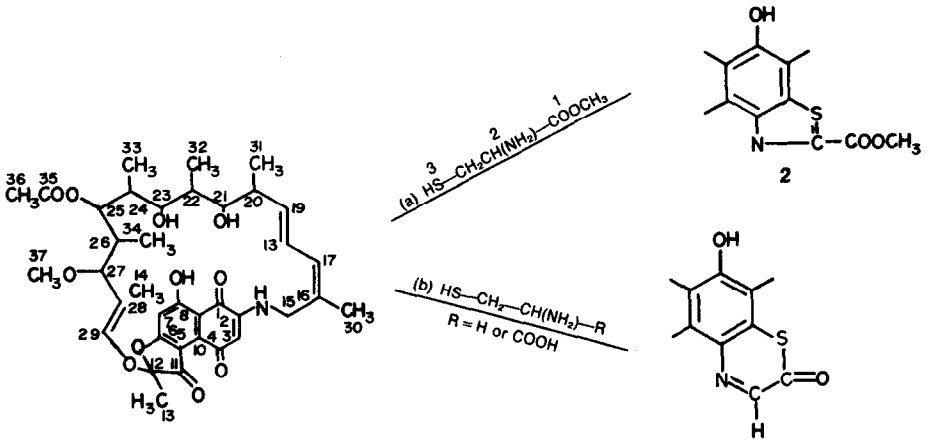
In the first paper of this series[1] it has been shown that by allowing an excess of rifamycin S, **1**, to react: (a) with cysteine methyl ester and (b) with cysteine or cysteamine, rifamycin derivatives **2** and **3** have been obtained (Scheme 1).

None of the two reactions gave the expected compounds, i.e. the rifamycins containing a 1,4-thiazine ring attached to position 3 and 4 of the chromophore. In case (a), in fact, a ring contraction thiazine–thiazole occurred. This result is not surprising because several examples of this contraction have been reported in literature.[2,3] To date no mechanism has been proposed, although it has been suggested to procede *via* the rearrangement of an intermediate peroxide.[4] In case (b) the formation of a 1,4-thiazin-2-one occurred and this result is new. Previous examples show the synthesis of benzo-1,4-thiazin-3-one or of dimeric compounds from the reaction between p-quinone and cysteine.[5] A reaction sequence between **1** and 2-amino-ethanethiol derivatives **4**, HS–CH_2–$CH(NH_2)$–R, is proposed in Scheme 2; it can account for the ring-contraction thiazine–thiazole and for the formation of the thiazin-2-one ring.

The nucleophilic attack of **4** (R=H, cysteamine; R=COOH cysteine; R=$COOCH_3$ cysteine methyl ester) on **1** gives compound **5** which is transformed through oxidation and cyclisation to the quinonimine **7** which rearranges to the more stable compound **8**. Addition of water to **8** gives compound **9** which is oxidized to the quinone like hemithoketal **10**.

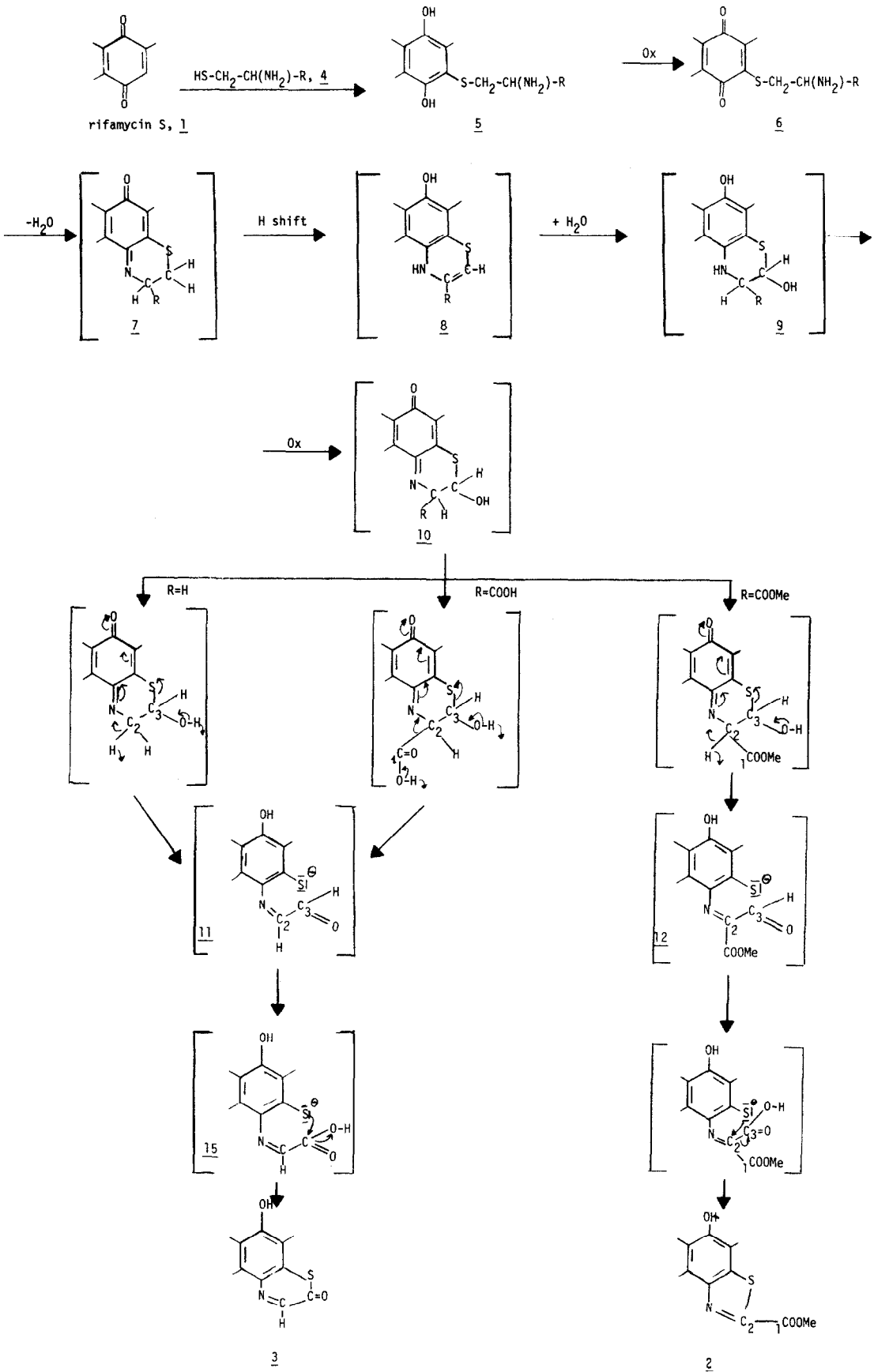
Rearrangement of **10** to a more stable hydroquinonic form leads, through cleavage of the S–CHOH bond and formation of the open intermediates **11** and **12**, to rifamycins **2** and **3** depending on the chemical nature of the substituent R. In fact, if R=H or COOH, the nucleophilic attack of S^- is on C-3 thus giving rise to the benzothiazin-2-one system. If R=COOMe, the nucleophilic attack of S^- is on C-2 because this latter is more electron deficient than C-3; the attack on C-2 with simultaneous elimination of C-3 gives the thiazole ring.

Support for the mechanism shown in Scheme 2 was



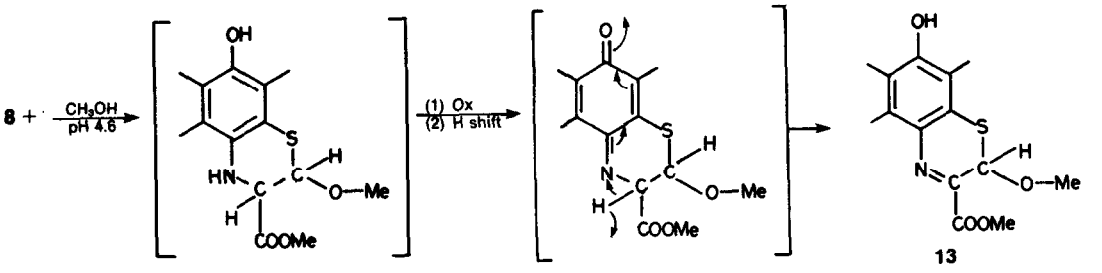
Rifamycin S, 1

Scheme 1.

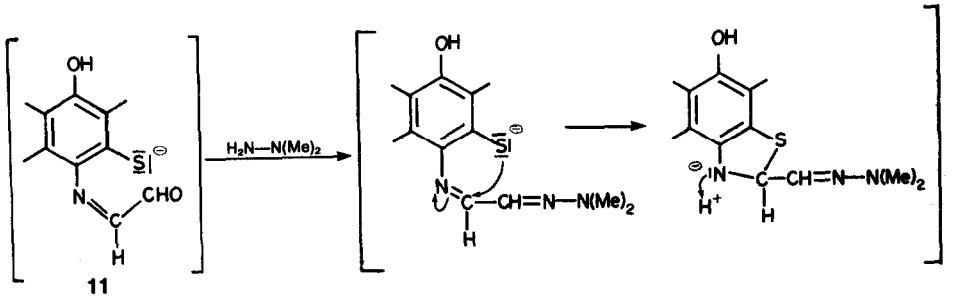

rifamycin S, 1
HS-CH2-CH(NH2)-R, 4
5
Ox
6
-H2O
7
H shift
8
+ H2O
9
Ox
10
R=H
R=COOH
R=COOMe
11
12
15
3
2

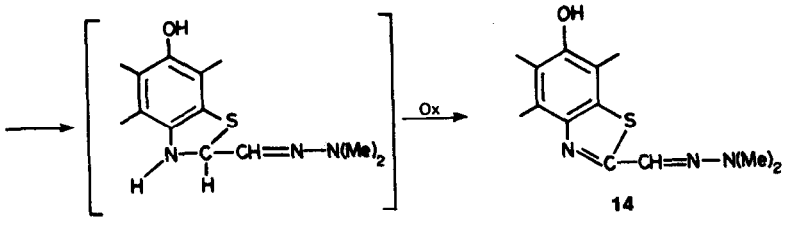
Scheme 2.

obtained experimantally; in fact, the isolation of **13** obtained from the reaction of **1** and systeine methyl ester, by the addition of methanol to compound **8** confirms the postulated formulation of **8**, i.e. the oxidation of CH_2 to CHOH:

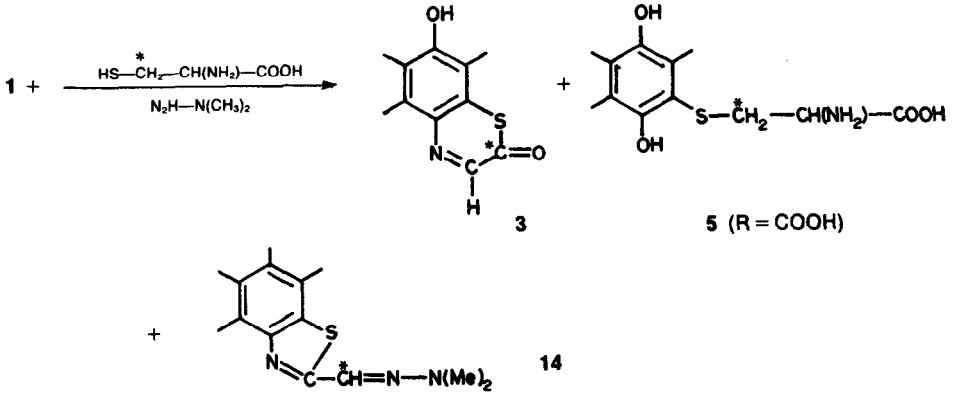


The isolation of compound **14** obtained by reacting **1** with cysteine or cysteamine in the presence of the trapping agent N,N-dimethyl-hydrazine,[6] confirms the postulated formation of **11**, i.e. the oxidation of CHOH to CHO:

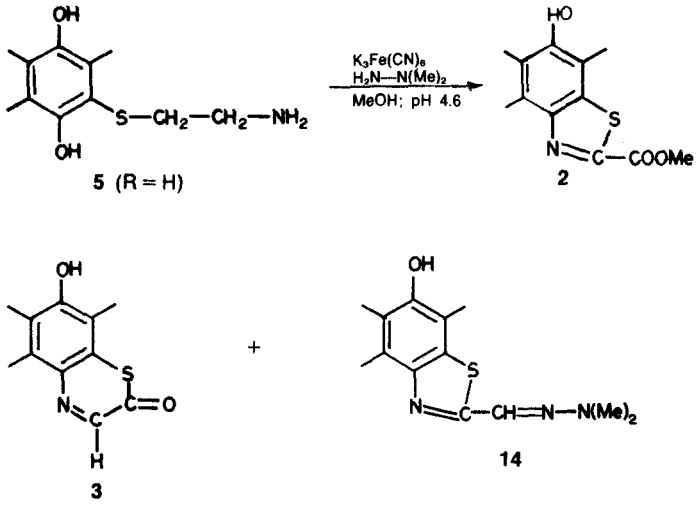




By reacting **1** with 3-^{14}C cysteine in the presence of N,N-dimethylhydrazine, compounds, **3**, **5** and **14** have been isolated. Their specific radioactivity was the same as labeled 3-^{14}C cysteine used as the starting material:



The isolation of compounds **2**, **3** and **14** obtained by oxidizing **5** (R=H) with $K_3Fe(CN)_6$ in the presence of N,N-dimethylhydrazine, in slightly acidic methanol confirms the postulated formation of **15**, i. e. the oxidation of CHO to COOH:



The isolation of the compounds obtained by allowing **1** to react with 3-^{14}C cysteine methyl ester hydrochloride (Table 1) showed that rifamycins **5** and **13** possess the same specific radioactivity of the starting 3-^{14}C cysteine methyl ester, while rifamycin **2** is not labeled, confirming that the ring-contraction thiazine–thiazole occurs with extrusion of the CH_2 group, which is lost as CO_2.

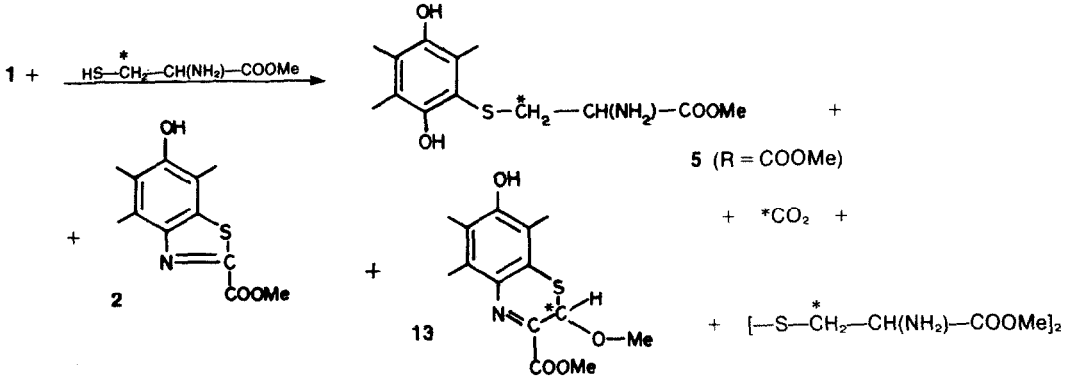


Table 1.

Compound	Found Amount mg	uM	uCi	uCi/mM
3-^{14}C cysteine methyl ester				471
5 (R=COOMe)	11.1	13.3	6.24	465.4
13	11.0	13.0	6.46	495.0
2	75			
3,3'-^{14}C cystine dimethyl ester	12.1	45.3	42.7	940
$^{14}CO_2$			162.0	

EXPERIMENTAL

The radioactive 3-^{14}C-cysteine was purchased from Radiochemical Centre, Amersham. The radioactivity of the samples was determined by liquid scintillation counting method in a Philips liquid spectrometer analyzer.

The samples were prepared by adding to 10 ml of Insta-gel (Packard) a soln of 10–20 ug of compound in 0.1 ml of MeOH using an internal standard for the determination of the quencing factor. Radiochemical purity was established using thin layer chromatography on pre-coated Merck silicagel 60 F 254 glass plates then followed by subsequent scanning with a Packard Scanner Model 7201.

^{1}H-NMR spectra at 60 MHz were obtained on a Varian A 60 D spectrometer, in $CDCl_3$ soln with TMS as the internal reference. UV–VIS spectra were measured on a Perkin–Elmer 4000 spectrometer. Analytical tlc's were carried out on silicagel Merck HF_{254} plates, using $CHCl_3$/MeOH 9:1 as the mobile phase. Column chromatography was performed with 0.05–0.20 mm silicagel Merck.

Rifamycin derivative **14**. A soln of 7 g of **1** (0.01 m), 0.600 g (0.005 m) of cysteine hydrochloride [or 0.565 g (0.005 m) of cysteamine hydrochloride] and 0.600 g (0.01 m) of N,N-dimethylhydrazine in 600 ml of MeOH and 20 ml of buffer pH 4.6 was left at room temp for 1 hr, then taken to dryness under vacuum. The purification of the crude material was carried out by column chromatography, eluting with $CHCl_3$/MeOH 98:2. Fractions containing pure compound **14** were pooled and taken to dryness. The residue crystallized from ETOAC (0.900 g). Yellow crystals, m.p. 172 dec. The ^{1}H NMR spectrum shows, together with the common signals of rifamycins, a singlet at δ 3.26 (6 H, N–CH_3)$_2$ and a singlet at δ 7.23 (1 H, CH=N).

UV–VIS spectrum in phosphate buffer (pH 7.38) λ max 225 nm (ϵ 43,700) 265 (26,600); 322 (24,500); 394 (23,300). Anal. ($C_{41}H_{52}N_4O_{11}S$) C, H, N, S Rf = 0.55.

Reaction among **1**, 3-^{14}C *cysteine and* N,N-*dimethylhydrazine*. A soln of 0.350 g of **1** (0.005 m), 0.030 g of cysteine hydrochloride (specific activity 0.432 mCi/mM) (0.0025 m) and 0.030 g (0.005 m) of N,N-dimethylhydrazine in 15 ml of MeOH and 1 ml of buffer pH 4.6 was left at r.t. for 1 hr, then taken to dryness uv. The purification of the crude material was carried out by preparative TLC ($CHCl_3$/MeOH 9:1). Radiolabeled compounds **3**, **5** and **14** were identified by comparison with authentic samples.

Reaction between **1** *and* 3-^{14}C *cysteine methyl ester hydrochloride*. 3-^{14}C cysteine methyl ester hydrochloride (specific activity 471.7 uCi/mM) was prepared by dissolving a mixture of 0.53 mM of cysteine and 0.0048 mM of 3-^{14}C cysteine (260 uCi) in 5 ml MeOH saturated with gaseous HCl, keeping the soln at 45° for 4 hr and taking it to dryness uv. The residue was dissolved in 2 ml MeOH and added to a soln of 0.700 g (0.001 m) of **1** in 30 ml MeOH and 2 ml AcOH buffer pH 4.6. This stirred soln was keeped under N_2 for 6 hr at r.t. and the evolved gas was collected in a soln of NaOH 1 N, then taken to dryness uv. The purification of the crude material was carried out by preparative tlc ($CHCl_3$/MeOH 9:1). Radiolabeled compounds were identified by comparison with authentic samples.

Oxidation of **5** (R=H) *with* $K_3Fe(CN)_6$ *in presence of* N,N-*dimethylhydrazine*. 0.660 g (0.002 m) of $K_3Fe(CN)_6$ were added to a soln of 0.780 g (0.001 m) of **5** (prepared according to Ref. 7) and 0.060 g (0.001 m) of N,N-dimethylhydrazine in 60 ml MeOH and 20 ml buffer pH 4.6. After standing 2 hr at r.t., the mixture was diluted with water, acidified to pH 3 with dil HCl, extracted with EtOAC and the organic phase taken to dryness uv. The purification of the crude material was carried out by preparative tlc ($CHCl_3$/MeOH 9:1); the compounds were identified by comparison with authentic samples.

Rifamycin derivative **13**. **13** has been isolated by preparative tlc from the crude material obtained by reacting rifamycin S and cysteine methyl ester as previously described.[1] The yield is about 100 mg from 7 g of rifamycin S. The ^{1}H NMR spectrum shows, together with the common signals of rifamycins a singlet at δ 3.35 (3 H, –C–O–CH_3), a singlet at δ 4.11 (3 H, –$COOCH_3$) and a singlet at δ 5.72 (1 H, –C(O–CH_3)–H).

Compound **13** rearranges to rifamycin derivatives **2** in aqueous acidic soln.

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