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A NOVEL RIFAMYCIN POSSESSING A DIHYDROQUINAZOLINIUM STRUCTURE[1]

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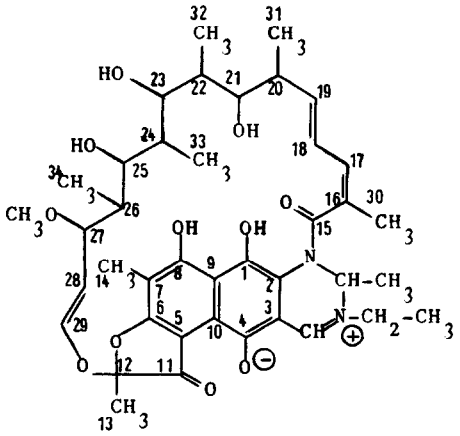
During the studies on oxidative cleavage of 3(N-substituted)aminomethyl rifamycins[2] as a part of our investigation on rifampicin[3], a deep-blue rifamycin was isolated, whose structure elucidation is the subject of the present note. As the product formerly obtained[2] showed low solubility in most solvents thus preventing the determination of helpful data, its more soluble desacetyl-derivative[4] was used for structural studies. Structure I can be assigned to the new rifamycin on the basis of chemical and physico-chemical evidences.



I

0.7 g of I was obtained by oxidation with MnO_2 of 10 g of 25-O-desacetyl-3(diethylaminomethyl)rifamycin SV (II)[4] in tetrahydrofuran to its quinone (Mn^{II} salt) and subsequent disproportionation in CCl_4-AcOH anhydrous solution under N_2, as already described[2]. It was crystallized twice from ethyl acetate and dried under vacuum at 60°C for one week. The product is very soluble in $CHCl_3$, alkanols and pyridine. Its water solubility is appreciable only at extreme pH values, suggesting amphoteric properties.

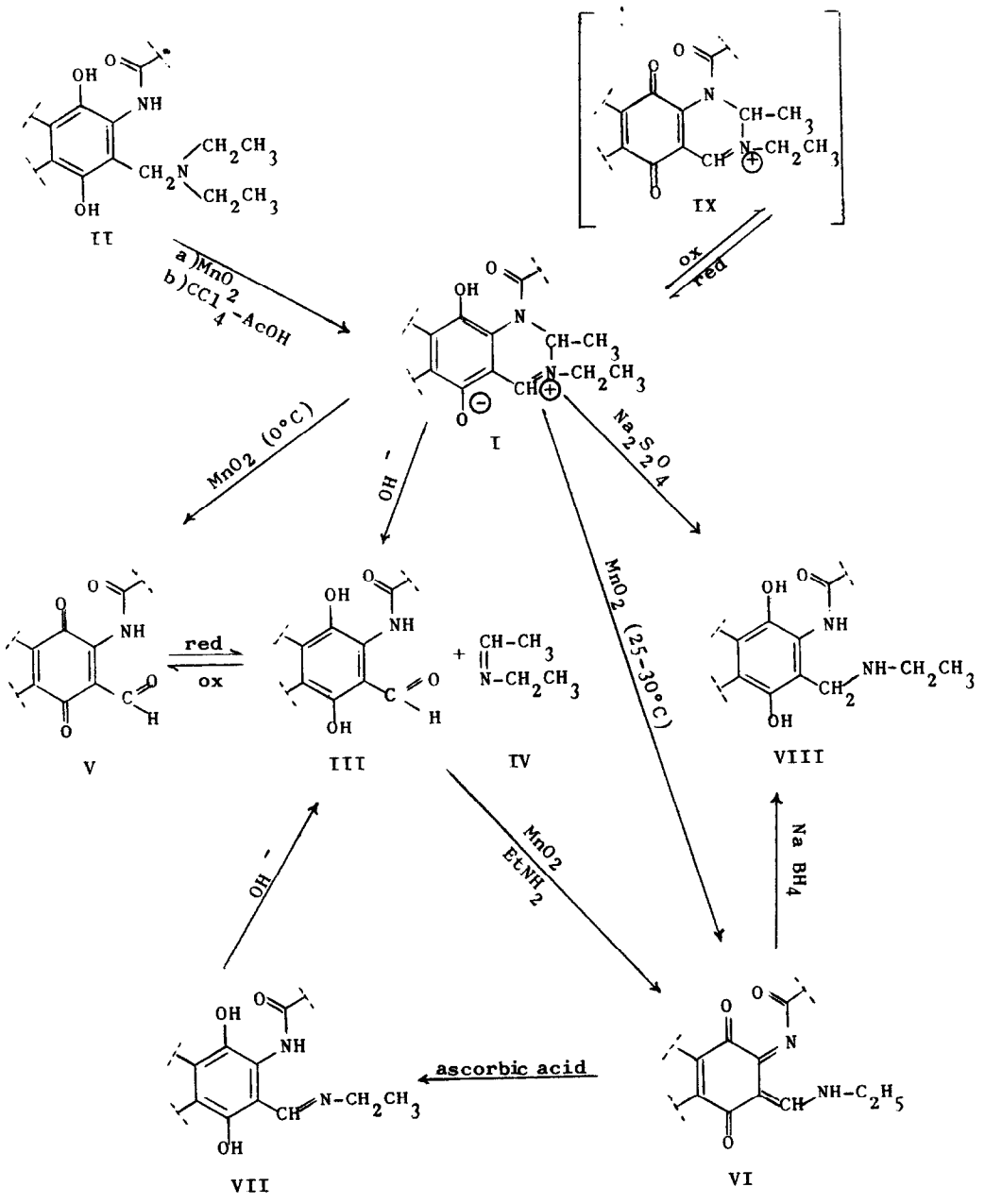

II
a) MnO2
b) CCl4 -AcOH
IX
ox
red
I
MnO2 (0°C)
OH -
Na2S2O4
MnO2 (25-30°C)
V
red
ox
III
IV
VIII
Na BH4
MnO2
EtNH2
OH -
ascorbic acid
VII
VI

Chart I

The chemical reactions hereafter described are shown in Chart I. Hydrolysis of I in a dioxane-0.5N NaOH (3:1) mixture at room temperature gave 25-O-desacetyl-3-formyl rifamycin SV (III), identified by comparison with an authentic sample[4]. This transformation, performed at a concentration of 100 μg/ml in a pH = 10 buffered solution, was spectrophotometrically found to be quantitative. Moreover, gas-chromatographic examination of the hydrolysis products, performing the reaction in a tetrahydrofuran-0,1N NaOH mixture (1:1), showed the presence of ethyl-ethylideneamine (IV) in the stoichiometric amount, indicating that acetaldehyde and ethylamine, or IV itself, are formed during the hydrolysis of I to III.

Cautious oxidation of I with different oxidizing agents such as MnO_2 and $K_3[Fe(CN)_6]$, performed in neutral solution at 0°C, gave 25-O-desacetyl-3-formyl-rifamycin S (V) as main product. The identity of V was established by mild reduction (ascorbic acid) to III. By performing the same oxidation at 25-30°C, the ethylimino-derivative of V was obtained. It was found identical to the reaction product of V with ethylamine and for it the tautomeric structure VI is to be preferred on the basis of NMR data. VI was reduced with ascorbic acid to the ethylimino-derivative of III (VII), which, by alkaline hydrolysis, yielded III as expected.

Reduction of I in ethanol-water (1:1) by $Na_2S_2O_4$ gave a yellow rifamycin (yield: 60%), to which structure VIII could be assigned on the basis of spectroscopic, polarographic and ionisation studies. Its identity was confirmed by comparison with an authentic sample obtained by reducing VI with $NaBH_4$ in ethanol.

The elemental analysis of I (C,H,N) gave the formula $C_{40}H_{52}N_2O_{11}$. Electronic spectrum of I (phosphate buffer pH 6.0) shows absorption maxima at 227 mμ (log ε = 4.60), 274 mμ (log ε = 4.29), 306 mμ (log ε = 4.22), 355 mμ (log ε = 4.23) and 565 mμ (log ε = 4.11), indicating a more conjugated chromophore, in respect to the known rifamycins. The ipsochromic shift of the 565 mμ maximum at low pH values points to the presence in I of a strong acidic function affecting the chromophore, analogously to the hydroquinonic rifamycins till now described[5]. The pK_a of this function could be spectrophotometrically calculated as about 0.2. A bathochromic shift of the 565 mμ maximum is observed at alkaline pHs, but decomposition takes place at the concentration used preventing any calculation. Potentiometric titration of I in EtOH-H_2O (70:30) with NaOH 0.1N revealed an ionizable group with pKa = 10.2 (eq. weight:720). From the above results, it fol-

lows that the product exists as internal salt. The basic group is formulated as the immonium form, in agreement with literature data[6] and NMR spectral data. This basic function can be titrated in anhydrous acetic acid with $HClO_4$ (eq. weight: 750).

In MeOH-acetate buffer pH = 5.4 (3:1) solution I shows a polarographic oxidation wave (2 electrons) in two steps with E1/2 = +0.07 and +0.20 volt vs. S.C.E., corresponding to the hydroquinonic moiety of rifamycins[7]. The occurrence of the process in two steps is interpreted as due to the non-equivalence of the hydroquinonic hydroxyls, one of which is ionized. In the same conditions a polarographic reduction wave (2 electrons) in two steps is observed, with E1/2 = -0.82 and -0.98 volt vs. S.C.E. This wave is attributed to the reduction of the immonium group, which occurs in two steps, analogously to the oxidation process.

NMR spectrum of I (fig. 1) shows one CH_3 doublet (8.56τ, J=6.8c/s) and one CH quartet (3.45τ, J=6.8c/s) due to the $CH-CH_3$ group, one $CH=\overset{\oplus}{N}<$ singlet (0.98τ) and one ethyl CH_3 triplet (8.37τ, J=7.2c/s), whose corresponding CH_2 quartet is in the range 6.5-5.9τ overlapped by other signals. The other peaks of the spectrum are consistent for a desacetyl-rifamycin structure[4] with the exception of the absence of the amidic NH signal and the accumulation of the "ansa" methyls within a narrower range. The latter fact is interpreted as due to the change of the "ansa" conformation induced by the participation of the amidic nitrogen to the dihydropirimidinium ring. Finally, two proton signals are shown at very low field (-4.9 and -6.4τ) which are ascribed, one to the association C_8-OH····O-C_1 and the other one to the C_1-OH····O=C_{15} association, due to the more favourable orientation of the amidic carbonyl. Thus, differently from the other rifamycins[8], the acidic function of I is attributed to the C_4-OH, whose strong ionisation is determined by the ortho $CH=\overset{\oplus}{N}<$ group. In the IR spectrum of I the νN-H amidic band, usually observed in the 3400 cm^{-1} region[8], is absent. Moreover, the furanone νC=O at about 1660 cm^{-1} is consistent with the hydroquinonoid formulation of I, in accordance with our observations in the rifamycin series[9].

The quinonoid form of I (IX) is rather unstable. Attemps for isolating IX, by oxidation of I in acidic solution, were unsuccessful. Anyhow, the formation of IX was observed by thin-layer chromatography of a solution of I in 0.02N tetrahydrofuran H_2SO_4 oxidized with MnO_2 in excess. The reversible reduction of IX to I via ascorbic acid was achieved and detected analogously. This finding rules out the possibility for I to be in the alternative 1,3,2-

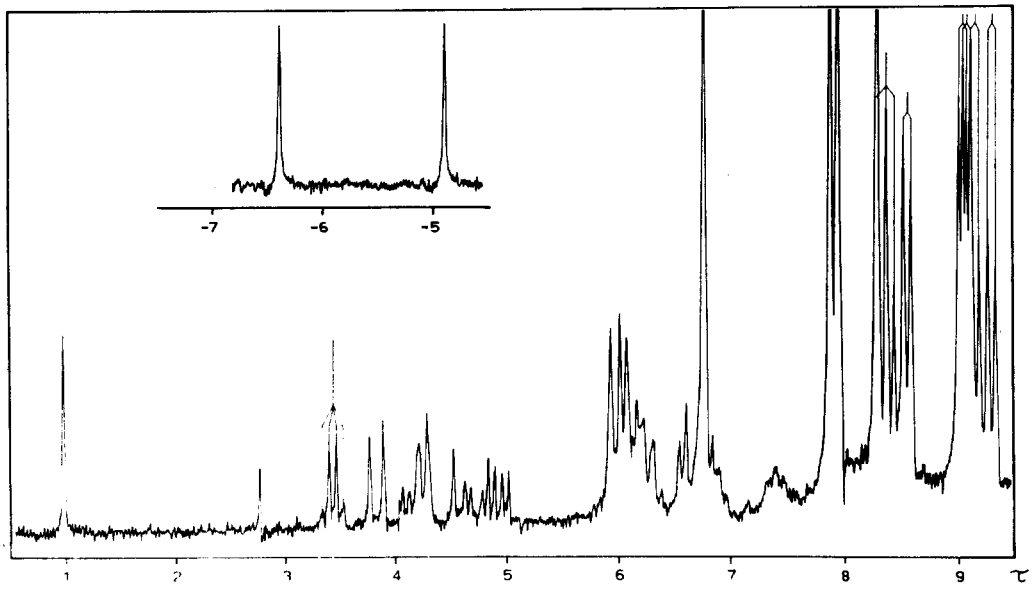


Fig. 1 - NMR spectrum of I at 100Mc/s in $CDCl_3$ (internal TMS, τ=10,00ppm).

oxazine form, involving the OH at C-4.

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