NOTES

RIFAMYCIN G, A FURTHER PRODUCT OF *NOCARDIA MEDITERRANEI* METABOLISM

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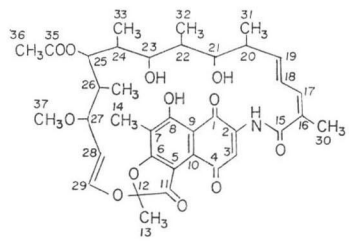
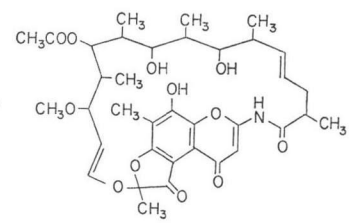
Several natural rifamycins have been isolated in addition to the products of *Nocardia mediterranei* fermentation originally described by SENSI and coworkers.[1] These include rifamycin SV[2] and rifamycin W[3] obtained from mutant strains of *N. mediterranei*, rifamycin L[4] a microbial transformation product of rifamycin S and rifamycin O[5] produced by a *Streptomyces* isolated from a soil sample; the production of rifamycin SV from a *Nocardia* isolated from soil was also reported.[6]

We wish to report the isolation of rifamycin G, a further product of *N. mediterranei* metabolism. Evidences for its origin from rifamycin S are also given and a structure based on physico-chemical determinations is proposed (Fig. 1).

Fig. 1



Rifamycin S



Rifamycin G

Isolation and Analytical properties

Rifamycin G is produced together with rifamycin complex in normal fermentations (for conditions and media see for instance[4]) but it first escaped notice since it is biologically inactive.

It was later noticed in extracts because of its ultraviolet absorption at 383 nm and was obtained as a crude material by:

a) Extraction of the filtered acidified broth with $CHCl_3$.

b) Separation from rifamycin complex by reextraction in buffer pH 7.38.

c) Acidification and extraction with ethyl acetate.

d) Evaporation of the solvent and precipitation in hexane.

Purification was accomplished by counter current distribution (solvent system: 0.07 M phosphate buffer-ethyl acetate) and crystallization from 95 % ethanol.

Rifamycin G is a white crystalline powder m.p. 250~252°C; u.v. absorption (in phosphate buffer pH 7.38): λ_{max} 268 nm ($E^{1\%}_{1cm}$ 338), λ_{max} 365 nm (shoulder), λ_{max} 383 nm ($E^{1\%}_{1cm}$ 234); two acidic functions with pKa values of 3.1 and 10.3 were calculated from variation of u.v. absorption with pH. It analyses best for $C_{38}H_{47}NO_{12}\cdot H_2O$ (M.W. 703.741, in agreement with the molecular ion in mass spectrum at m/e=685).

Origin from Rifamycin S

Rifamycin G appears to be a metabolic derivative of rifamycin S. The latter is in fact converted into both rifamycin B[4] and rifamycin G by washed mycelium of *N. mediterranei*, as shown by the data reported in Table 1. The presence of barbital in the medium appears to increase the conversion into rifamycin B, but to have no effect on rifamycin G yields. Evidence that rifamycin G does not derive from *de novo* synthesis was obtained by adding to washed mycelium suspensions C^{14} labelled rifamycin S (100 mg, 5.8×10^5 dpm/m mole). After incubation rifamycin G was recovered (27 mg) having a specific activity of 3.6×10^5 dpm/m mole.

Table 1. Transformation of rifamycin S into rifamycins B and G by washed *N. mediterranei* mycelium

Barbital in growth medium	Barbital in resuspension medium	Rifamycin S added (μg/ml)	Rifamycin B formed (μg/ml)	Rifamycin G formed (μg/ml)
−	−	—	8	17
−	−	200	70	77
−	+	—	13	10
−	+	200	89	62
+	+	—	16	10
+	+	200	114	76

Washed mycelium of *Nocardia mediterranei* was prepared and resuspended in 0.07 M phosphate buffer pH 6.5 as previously described.[4] The media differed for the presence (+) or absence (−) of 1 g/liter of sodium diethylbarbiturate. Where indicated 200 μg/ml of rifamycin S were added to the resuspended mycelium. After 20 hours of incubation at 28°C the products were analyzed. Rifamycin B was determined by a spectrophotometric differential method[7] and rifamycin G was estimated spectrophotometrically after extraction and counter-current distribution.

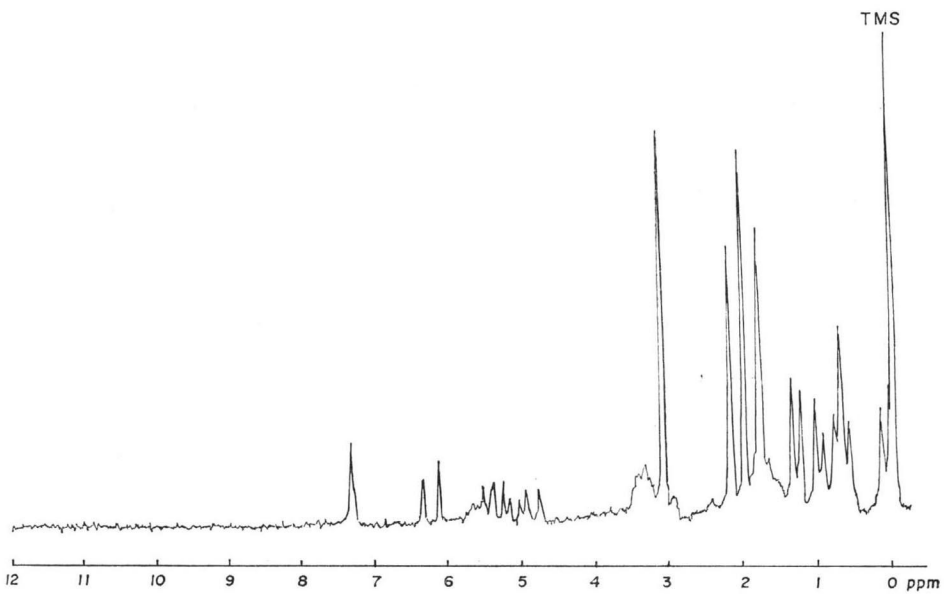
Structural Considerations

Elemental analysis and high resolution mass spectrometry show that rifamycin G has two hydrogen atoms more and one carbon atom less than its parent rifamycin S.

Comparison of ^{1}H NMR spectra of the two compounds (Fig. 2; for rifamycin S ^{1}H NMR spectrum see[8]) established that the two extra hydrogen atoms are located on carbon 16 and 17. In fact the saturation of the 16—17 double bond of rifamycin S is demonstrated by the disappearance of the singlet (3H) at σ=2.01 (attributed to methyl 30 at C-16) and the appearance of a new doublet (3H) at δ= 1.30. Moreover the signal of the hydrogen at C-17 is no more present in the region δ= 6.0~6.5 and the signals corresponding to hydrogens on carbon 18 and 19 (that are now on a isolated double bond) are shifted upfield at δ=5.4~5.8.

Substantially unchanged are the chemical shifts of the signals corresponding to methyl 37 (methoxy at C-27, δ=3.10) methyl 36 (acetoxy at C-25, δ=2.02), hydrogens on double bond 28-29 (δ=5.25 and δ=6.29), hydrogens on oxygen bearing carbons 25 (δ=4.87), 23 (δ=3.04), 21 and 27 (δ=3.2~3.6). This suggests that no other variation occurred in the ansa chain structure; moreover the chemical shifts of the methyls 31, 32, 33, 34 (doublets from δ=0.1 to 1) demonstrate that, as in rifamycin S the ansa chain is shielded by

Fig. 2. ^{1}H NMR spectrum of rifamycin G.
The spectrum was recorded at 60 MHz in $CDCl_3$, concentration 3×10^{-2} M, PFT NMR, Bruker WT60.



the aromatic ring and thus still spans it. This is supported also by the IR spectrum where the peaks corresponding to ν C=O of the acetyl group at 1720 cm^{-1} and to the second amide band δ N-H at 1510 cm^{-1} could be identified.[9]

The u.v. spectrum indicates a major modification in the naphthoquinone moiety, the so-called chromophore. Comparison of the electron impact mass spectrum of rifamycins G and S[10] revealed that the high intensity peak at *m/e* 273 ($C_{14}H_{11}NO_5$) of rifamycin S attributed to the chromophoric ion was replaced by a peak at *m/e* 261 ($C_{13}H_{11}NO_5$). Conversion of rifamycin S to rifamycin G thus involves the loss of one carbon atom in the chromophore. No variation in respect to rifamycin S appears to have occurred in the aromatic and furanone rings including carbon atoms from 5 to 12. In fact ^{1}H NMR signals of methyl 14 (δ=2.20) and methyl 13 (δ=1.80) are substantially unchanged. In the I.R. spec trum the ν C=O of furanone carbonyl is still present at 1725 cm^{-1}. The hydroxyl group on carbon 8 appears (as usual in rifamycins) to be responsible for the acidity of the molecule: on treatment with diazomethane a monomethyl derivative is obtained (m.p. 248~250°C, analysis and molecular ion in agreement for $C_{37}H_{49}NO_{12}$) in which only the weak acidic function (pK 10.5) is retained and whose ^{1}H NMR shows a signal (singlet, 3H) at δ=4.10 superinposable with that attributed to the aromatic methoxy of 8-methyl rifamycin S.

One of the carbon atoms 1 to 4 of rifamycin S is thus missing in rifamycin G. Several evidences indicate that it might be the carbon atom 1; in fact the carbonyl group in this position is no more present in rifamycin G since: a) the molecule lacks oxido-reduction properties; b) in rifamycin S the hydroxyl group in position 8 gives a strong hydrogen bond with the carbonyl group in position 1 as shown by a signal at δ 12.51 in ^{1}H NMR. No such signal is present in rifamycin G spectrum; c) the lack of this hydrogen bond explains the lower pKa value (3.1) of rifamycin G in comparison with rifamycin S (pKa=7.2); d) in ^{1}H NMR spectrum the signal attributed to the hydrogen on carbon 3 is shifted from δ 7.77 of rifamycin S to δ=7.35 in rifamycin G. This suggests that in the latter it is no more in β position to a carbonyl group.

In conclusion, the structure reported in Fig. 1 in which the quinone ring of rifamycin S is replaced by a γ-pyrone ring is consistent with all the data above discussed and is thus proposed for rifamycin G.

Acknowledgement

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