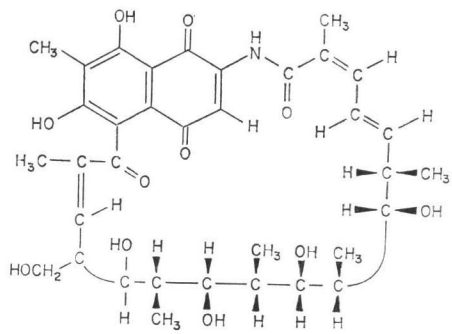
IDENTIFICATION AND PREPARATION OF DAMAVARICINS, BIOLOGICALLY ACTIVE PRECURSORS OF STREPTOVARICINS[1]

Sir:

MARTINELLI, *et al.*,[2] recently described a new metabolite obtained from a mutant of *Nocardia mediterranei*, which normally produces rifamycin B. The Lepetit group have assigned the structure below to the metabolite, which they named rifamycin W,* and have indicated that rifamycin W is converted biologically to rifamycin B.[3] We describe here damavaricin D (DmD, **1**), a similar metabolite from *Streptomyces spectabilis* which is biologically active, and demonstrate that DmD and other damavaricins can be synthesized chemically from the streptovaricins.

Damavaricin D($C_{37}H_{47}NO_{12}$,** dec. >290°C) was isolated by repeated chromatography of streptovaricin complex over silica gel. The molecular formula showed the compound to be closely related to the streptovaricins, lacking three carbons and one oxygen of streptovaricin D(SvD). The missing atoms were identified as the enol acetate (from the ir spectrum, which lacked the characteristic absorption at 1760 cm^{-1}; the pmr spectrum, which lacked one deshielded methyl in the 2.0~2.3 ppm region; and the cmr spectrum ($CDCl_3$), which lacked acetate carbonyl and methyl carbons at 169 ppm and 21 ppm, respectively) and the methylenedioxy group [from the absence of a deshielded methylene in the pmr and cmr spectra, near 5.7 (AB quartet) and 9.0 ppm, respectively]. Replacement of a methylenedioxy (—OCH_2O—) group of SvD by a phenol and enol (-OH HO-) in DmD and of an enol acetate (-$OCOCH_3$) by an enol (-OH) would account for loss of C_3H_2O, whereas C_3H_4O is in fact missing. The remaining two hydrogens are (formally) lost by conversion of a hydroquinone to a quinone (identified by carbonyl absorption in the infrared spectrum of DmD at 1640 and 1620 cm^{-1} and in the cmr spectrum at 184.4 and 184.9 ppm). In addition, the quinone methide of SvD has (formally) tautomerized to a *p*-hydroxyaroyl group in DmD. The hydroxyl at C-21 is established by the characteristic low field proton absorption ofa *peri*-hydroxy naphthoquinone, in DmD at 12.50 ppm in the pmr spectrum. The acyl group (C-17) of DmD is masked in the infrared spectrum (1640 cm^{-1}) due to hydrogen bonding with the C-19 hydroxyl (the carbonyl of *o*-hydroxyacetophenone is found at 1640 cm^{-1}),[4] but is observed in the cmr spectrum at 202.0 ppm (*o*-hydroxyacetophenone, 204.4 ppm).[5] The C-19 hydroxylic proton of DmD is observed at 9.69 ppm (DMSO-d_6). Aliphatic protons and carbons of the metabolite are found at nearly identical



Rifamycin W

positions to those of SvD.[1,6] Thus, the structure **1** can be assigned to DmD.

Damavaricin D can also be obtained by chemical conversion, from SvD. Treatment of SvD with oxygenated concentrated ammonia - methanol (1:1) at room temperature for 3 hours gave a mixture of two compounds-DmD, identified by spectral comparison with a sample from natural sources, and atropisodamavaricin F_D, aDmF_D, **2**, the corresponding atropisomeric C-7 lactone [$C_{36}H_{43}NO_{11}$,* mp 218~220°C, $[\alpha]_D^{25}$—680° (*c* 0.050, $CHCl_3$)]. The structure of the latter compound was assigned from its pmr spectrum, which lacked the methoxyl group as well as the methylenedioxy group and enol acetate methyl of SvD. Assignments to the natural or atropiso series

* Rifamycin W is actually more closely related to the streptovaricins than to the previously reported rifamycins.

** Microanalyses and low resolution mass spectral data are in agreement with the molecular formulas assigned.

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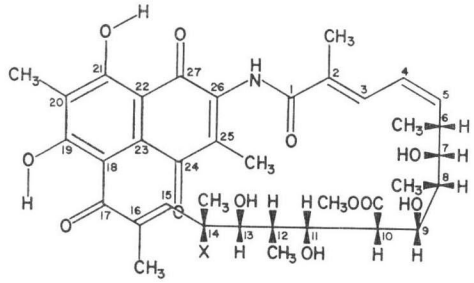
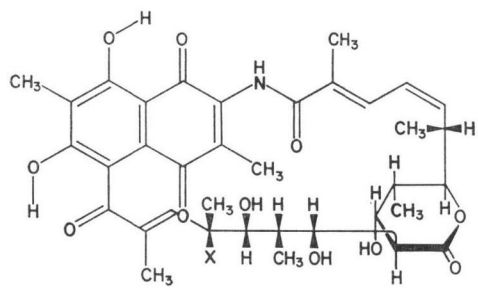


1 (DmD) : X=H
3 (DmC) : X=OH



2 (aDmFD) : X=H
4 (aDmFC) : X=OH

were on the basis of positive or negative rotation, respectively.[7]

Similar treatment of streptovaricin C(SvC) with oxygenated methanolic ammonia gave in 56 % yield damavaricin C [**3**, $C_{37}H_{47}NO_{13}$,* mp 278~280°C dec, $[\alpha]_D^{27}+806°$ (*c* 0.124, $CHCl_3$)] and atropisodamavaricin F_C[aDmF$_C$, **4**, $C_{36}H_{43}NO_{12}$,** mp 222~224°C, $[\alpha]_D^{25}-734°$ (*c* 0.112, $CHCl_3$)], with the former's pmr spectrum lacking the methylenedioxy and enol acetate groups, the latter's the methoxyl group as well.

The damavaricins retain much of the biological activity of the streptovaricins, as might be anticipated from their chromophoric resemblance to rifamycin S.[8] Thus, damavaricin C inhibits DNA-dependent RNA polymerase† to the extent of 47 % and RNA-dependent DNA polymerase (reverse transcriptase)† to the extent of 48 %, and inhibits the growth of selected bacteria (*Staphylococcus aureus*, *Sarcina lutea*, *Klebsiella pneumoniae*, *Mycobacterium avium*),† while damavaricin F_C inhibits RNA-dependent DNA polymerase to the extent of 59 %.

Isolation of damavaricin D from the fermentation mixture produced by *S. spectabilis* implicates it as a precursor to SvD, by a process involving O-methylation and subsequent methoxyl oxidation,[10] quinone reduction and subsequent phenol acetylation. Streptovaricin D is the principal streptovaricin produced in our biosynthetic studies[11] and we have now shown that it is a precursor to the other streptovaricins. Streptovaricin D labeled biosynthetically by [*methyl*-^{14}C]-methionine in the methoxyl, methylenedioxy, and 25-methyl groups was treated with a growing culture of *S. spectabilis*. Of the products isolated after 4 days' growth, SvD contained 77 % of the label and SvC 23 %. In a separate experiment [7-^{3}H]streptovaricin C, prepared by reduction of streptovaricin E with sodium borotritide, was treated with a cell-free system prepared from a culture of *Streptomyces spectabilis*. Radioactive products isolated from a thin-layer plate were SvA (6 % of label), SvB (24 % of label), and SvC (70 % of label.)

Thus, the sequence for streptovaricin biosynthesis appears to involve the sequence DmD→SvD→SvC→SvB→SvA. Other minor streptovaricins presumably are also formed from SvC (SvE, SvG, SvJ), or from SvB (SvK).

* Microanalyses and low resolution mass spectral data are in agreement with the molecular formula assigned.

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†Conditions employed for the biological studies were the same as those described previously.[9]

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