SCOPAMYCIN. II*

IDENTIFICATION OF THE SUGAR MOIETY OF SCOPAMYCIN A AS 2-*O*-METHYL-L-RHAMNOSE

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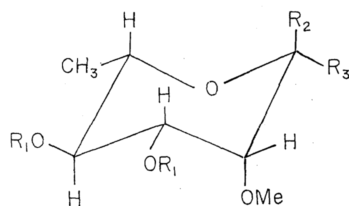
The antifungal agent scopamycin A has been the subject of further chemical study. On treatment with acid it yields a 6-deoxy sugar which has been determined by nmr spectroscopy and chemical synthesis to be 2-*O*-methyl-L-rhamnose (6-deoxy-2-*O*-methyl-L-mannose).

Scopamycin A, a metabolite of a strain of *Streptomyces aureofaciens* (ETH 28832), has been described in a preliminary way by HÜTTER *et al.*[1] Spectroscopic data[2] indicate that scopamycin A is a compound of high molecular weight ($C_{44}H_{74}O_{14}$) with a large number (*ca.* 7) of carbon-bound methyl groups, moderate unsaturation (at least three olefinic linkages, and two carbonyl groups) and few ring structures. These facts have led to the prediction[2] that scopamycin A may be a novel antibiotic of the macrolide class. If so, scopamycin A represents a new pattern of biogenetic origin and biological activity. It has no known biological acitivity against bacteria and a substantial portion of its structure appears to be derived from "propionate". The known biological activity of scopamycin A is restricted to a potent inhibition of the growth of fungi and yeasts. Members of the macrolide family of antibiotics with medium-size rings are usually formed biologically in part from "propionate"-derived substrates and they are primarily inhibitory to the reproduction of gram-positive microorganisms. Macrolides with larger lactone rings (*e.g.* nystatin) usually reflect the exclusive or predominant use of "acetate"-derived building blocks for their biogeneses and they usually display antifungal biological activity.



	R_1	R_2	R_3
I	H	OH	H
II	H	H	OH
III	CH_3CO	CH_3COO	H

Recently we reported[2] the results of a detailed nmr analysis of scopamycin A and its crystalline diacetyl derivative. One conclusion concerned the possible occurrence of a sugar moiety in the structure. The methyl doublet at 1.44 ppm (δ) in the spectrum of scopamycin A is collapsed by irradiation at approxi-

* Reference 2 is taken as Part I of this series.

Table 1. Nmr parameters of the 6-deoxy sugar of scopamycin A [a)]

	Chemical shifts [b)]			Coupling constants [c)]	
	α-Anomer	β-Anomer		α-Anomer	β-Anomer
H-1	5.76	5.17	$J_{1,2}$	1.8	1.0
H-2	3.99	3.99	$J_{2,3}$	3.5	—
H-3	4.66	—	$J_{3,4}$	9.2	—
H-4	4.11	—	$J_{4,5}$	9.2	—
H-5	4.51	—	$J_{5,CH3}$	6.0	6.0
CH_3-5	1.65	1.62			
O-CH_3	3.57	3.83			

a) Chemical shifts and coupling constants were confirmed by appropriate spin decoupling experiments.
b) Reported in ppm (δ) measured downfield from internal tetramethylsilane in pyridine-d_5 solution at ambient probe temperature.
c) Reported in Hz and determined from first order analysis.

mately 4.1 ppm and this is the only methyl group coupled to a proton resonating downfield of 3.0 ppm. This indicated that scopamycin A can contain not more than one 6-deoxy sugar. Such a sugar has now been isolated after acid hydrolysis of scopamycin A.

Initial fractions from silica gel column chromatography of the concentrated neutralized acid hydrolysate yielded after concentration, a water-white oil whose nmr spectrum indicated that in pyridine-d_5 it was a mixture of anomers of a 6-deoxy-aldohexose in the approximate ratio of 7:1. The chemical shifts and coupling constants determined for the two anomers are presented in Table 1.

The furthest downfield resonance of a proton in the major anomer (I) of the sugar from scopamycin A, attributable to the anomeric substituent, appears at 5.76 ppm whereas the analogous resonance of the minor component (II) appears at 5.17 ppm. From this difference in chemical shifts it is clear that the major component has its anomeric proton in an equatorial (α) configuration and that in the minor component this configuration is axial (β)[3)]. Both resonance patterns appear as doublets (major: $J_{1,2}$=1.8 Hz; minor: $J_{1,2}$=1.0 Hz) and because of the magnitude of the vicinal coupling constants it is concluded that H-2 has an equatorial orientation[4)]. In view of the known configurational dependence of vicinal couplings[5)], the observed magnitudes of the coupling constants are consistent with an equatorial-equatorial proton relationship in the major component and an axial-equatorial proton relationship in the minor component. In the case of the major component (I) the assignment of the resonance patterns produced by H-2, H-3, H-4 and H-5 was made by spin-decoupling experiments (Table 1). The large $J_{3,4}$ and $J_{4,5}$ values measured for the couplings show that H-3, H-4, and H-5 all have axial orientations. Taken with the equatorial orientations already demonstrated for H_1 and H_2 these results define the skeletal structure of the major anomer (I) of the sugar from scopamycin A as that of an α-rhamnose.

The resonance patterns of H-2, H-3, H-4, and H-5 in the minor anomer (II) are obscured by those of the ring protons in the major component (I). However, irradiation at 3.99 ppm, the chemical shift determined for H-2 in the major anomer (I), causes simultaneous collapse of the patterns assigned to the anomeric protons in

both (I) and (II). Thus the chemical shift of H-2 in the minor anomer (II) is approximately the same as that found for the more abundant isomer (I). The methyl group absorption pattern of the minor anomer (II) is a doublet (J_{5,CH_3}=6.0 Hz) found at 1.62 ppm.

In addition to the resonance patterns of the ring protons, the spectrum of the mixture of the anomeric sugars showed three-proton singlets at 3.57 and 3.83 ppm, which are attributable to a single methoxyl group present in each component (I and II, respectively). The anomeric sugars (I) and (II) are therefore *O*-methylrhamnoses. The position of the methoxyl group substitution was determined by a consideration of the nmr spectrum of a peracetyl derivative. The peracetylated mixture of sugars was fractionated by column chromatography using silica gel and the major anomer was obtained as an oil (III). Its nmr spectrum indicated that only a small amount (<5 %) of the peracetyl derivative of the minor anomer was present. In this spectrum the doublet attributable to the anomeric proton of the major anomer is at 6.48 ppm. This absorption is coupled to a one-proton triplet signal at 3.93 ppm which is thereby assigned to H-2. Since the chemical shift of H-2 is not changed significantly by the peracetylation, the methoxyl group must be located at C-2. The resonance pattern of H-5 can be assigned to the absorption pattern centered at 4.10 ppm which is coupled (J_{5,CH_3}=6.0 Hz) to the doublet methyl group signal at 1.26 ppm. By exclusion, the two proton multiplet absorption pattern centered at approximately 5.6 ppm is assigned to H-3 and H-4. The resonance signals of the methoxyl and the acetoxyl groups are observed at 3.44 ppm, 2.08 ppm ($2\times CH_3$) and 2.10 ppm ($1\times CH_3$) respectively.

Although the observations summarized above show that the sugar isolated from scopamycin A is a 2-*O*-methylrhamnose, they do not define its absolute configuration. A stereospecific partial synthesis of the new sugar from L-rhamnose completed the proof of structure. 2-*O*-Methyl-L-rhamnose was synthesized according to the method of JONES *et al.*[6] and had an nmr spectrum identical to that of the product (I and II) obtained by hydrolysis of scopamycin A. Acetylation of the synthetic product gave an oil having an infrared spectrum and optical rotation identical to that of the acetylated product (III) obtained from scopamycin A. Thus, the sugar obtained from scopamycin A by acid hydrolysis is 2-*O*-methyl-L-rhamnose.

The configuration of the glycosidic linkage between the sugar and the aglycon of scopamycin A can also be deduced from the nmr spectra available. The conclusion that this is an *alpha* (α) glycoside is supported by the following considerations. At ambient temperature in pyridine-d_5 solution the resonance of the anomeric proton of 2-*O*-methyl-L-rhamnose in scopamycin A is observed at 5.40 ppm. This proton has been designated in an earlier report[2] as H_S. The resonance of the anomeric proton in the spectrum of the free sugar (I and II) occur at 5.76 ppm (α-anomer) and 5.17 ppm (β-anomer). These latter assignments depend on the correlation reported by LEMIEUX[3]. As complex formation with pyridine-d_5 primarily involves free hydroxyl groups[7], in this solvent the anomeric proton resonance in the spectrum of a glycoside may be expected to occur at higher field than the analogous resonance in the spectrum of the free sugar. The resonance observed for the anomeric proton

Table 2. H_1-H_2 Coupling constants for 2-*O*-methyl-L-rhamnose derivatives

Compound	$J_{1a,2}$	$J_{1e,2}$
2-*O*-Methyl-L-rhamnose	1.0 Hz	1.8 Hz
2-*O*-Methyl-L-rhamnose triacetate	<1.0 Hz	1.5 Hz
Methyl-2-*O*-methyl-L-rhamnoside diacetate[12]	<1.0 Hz	1.5 Hz
Aranciamycin [12]	<1.0 Hz	
Scopamycin A		1.5 Hz

in the glycoside is upfield of the value found for the *alpha* form of the free sugar but downfield of that attributable to the *beta* form. Thus, in scopamycin A the 2-*O*-methyl-L-rhamnose moiety exists as an α-L-glycoside.

A confirmation of the assignment of configuration to the glycosidic linkage in scopamycin A is provided by comparing the coupling constants for the anomeric proton and H-2 in various 2-*O*-methyl-L-rhamnose derivatives (Table 2). The couplings ($J_{1,2}$) between H-1 (axial) and H-2 are always equal to or less than 1 Hz. The $J_{1,2}$ values between H-1 (equatorial) and H-2 are between 1.5 and 1.8 Hz. The magnitude of this vicinal coupling constant in scopamycin A when measured at high temperature in pyridine-d_5 solution is 1.5 Hz.

The assignment of an α-L-configuration to the glycosidic link in scopamycin A is further supported by the location at 5.07 ppm of the resonance band for the anomeric proton of scopamycin A when measured in deuterochloroform solution. This chemical shift is in close agreement with those found for the anomeric protons of other α-L-sugar glycosides[8].

This configurational assignment is in agreement with KLYNE's rule[9] which predicts for glycosides of common biogenetic origin the same absolute configuration at the anomeric centers (*i.e.* α-L or β-D). Originally confined to the field of cardiac glycosides, this prediction was later extended to include the case of macrolide antibiotics[8].

It should be noted that the configurational assignments based on our interpretation of the nmr spectrum of 2-*O*-methyl-L-rhamnose (I+II) is in direct contradiction to one following the rule recently proposed by SINCLAIR and SLEETER.[10] This rule predicts that the chemical shift of the methyl protons of the *alpha*-anomers of a 6-deoxy sugar should be *upfield* of that observed for the *beta*-anomer. As shown in Table 1, the chemical shift (CH_3-5) value of the *alpha*-anomer of 2-*O*-methyl-L-rhamnose (I) is at 1.65 ppm, which is *downfield* of the value of 1.63 ppm found for the *beta*-anomer. This discrepancy is not observed in the spectra of the triacetate of (I) or of the methyl glycoside diacetate derivatives.[11]

The sugar identified as a part of scopamycin A is relatively rare. Recently, 2-*O*-methyl-L-rhamnose has been found to be a part of the antibiotic aranciamycin[11]. It is rather surprising that the configuration indicated for the glycosidic link in aranciamycin is *beta* which is not only opposite to that proposed for the same sugar in scopamycin A but rare for sugars of the L-series. The mirror image form, 2-*O*-methyl-D-rhamnose, is produced by a strain of *Mycobacterium tuberculosis*[12] as well as by a *Myxobacterium* species.[13]

The number of oxygen atoms present in scopamycin A would allow a formulation with a second sugar moiety. However, such a sugar could not be a 6-deoxy sugar[2]. As these are the predominant form of sugars found in secondary metabolites of actinomycetes it is likely that scopamycin A is a monoglycoside.

Experimental

Nuclear magnetic resonance spectra were measured using a Varian HA-100 spectrometer and pyridine-d_5 solutions with tetramethylsilane as an internal reference. Optical rotations were measured at 589 nm in 1 dm cells, using a Rudolph Model 80 spectropolarimeter. Infrared spectra were measured using either liquid films (oils) or nujol mulls (solids) and a Beckman IR-12 spectrophotometer. Solutions were concentrated on a "Rotavapor" under reduced pressure at temperatures below 50°C. Microanalyses were determined by the Mid-West microanalytical service.

Hydrolysis of scopamycin A

Scopamycin A (560 mg) in methanol (10 ml) was treated with 5 N HCl (2 ml) at room temperature for 22 hours. The mixture was washed through a column of Amberlite MB-3 mixed-bed resin (1 cm × 20 cm) with methanol and the eluate was concentrated to a light yellow oil (472 mg). This oil was digested in benzene and chromatographed on a column of Whatman SG31 silica gel (1.5 cm × 41 cm). Initial benzene eluates were concentrated leaving a colorless oily residue (I+II) (19 mg) (Rf 0.20 on plates developed with ethyl acetate) having an ^{1}H nmr spectrum as described in the text.

Acetylation of I+II

The oil (I+II) (19 mg) in pyridine-d_5 (0.4 ml) was treated with acetic anhydride (0.2 ml) overnight at 25°C. The mixture was then poured into water (10 ml) and extracted with chloroform (5 × 2 ml). The combined extracts were concentrated and the oily residue was digested in, and concentrated from, 2,2,4-trimethylpentane several times to remove pyridine-d_5. The crude product was chromatographed on a column of Mallinkrodt silicic acid (1 cm × 29 cm), eluted with methylene chloride - ethyl acetate (5 : 1). Early fractions gave a colorless oil (III) (27 mg), $[\alpha]_D$ −51° (*c*, 1.4 $CHCl_3$).

Synthesis of 2-*O*-Methyl-L-Rhamnose

2-*O*-Methyl-L-rhamnose was synthesized according to the method of JONES *et al.*[2] except that the ethereal extract of the methylene derivatives was separated by column chromatography on Merck silica gel. Thin-layer chromatography indicated that initial fractions contained 1,2,3,5-di-*O*-methylene-L-rhamnofuranose. Subsequent fractions deposited crystals of 3,4-*O*-dimethyleneoxy-L-rhamnose from chloroform m.p. 132~132.5°C, $[\alpha]_D$ +12° (1.5 hours, *c* 0.9, H_2O) ν_{max} 3400 cm^{-1} (strong). Found: C 46.3, 46.5; H 6.9, 7.0 %. $C_8H_{14}O_6$ requires C 46.6, H 6.8 %.

Methyl-3,4-di-*O*-methyl-L-rhamnoside had m.p. 113~119.5°C, $[\alpha]_D$ +84° (*c* 1.2, H_2O) and was transparent above 3000 wavenumber in the infrared. Found: C 51.5, H 7.5 %. $C_{10}H_{18}O_6$ requires C 51.3, H 7.7 %.

2-*O*-Methyl-L-rhamnose obtained by acid hydrolysis of methyl-3,4-di-*O*-methyleneoxy-2-*O*-methyl-L-rhamnoside had an nmr spectrum identical with that of the sample obtained from scopamycin A.

Acetylation of the synthetic 2-*O*-methyl-L-rhamnose under conditions similar to those used for acetylation of the natural product gave an oil having an infrared spectrum identical with that of the acetylated natural product and having $[\alpha]_D$ −52° (*c* 2.7, $CHCl_3$).

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