# MICROBIAL CONVERSION OF AVERMECTINS BY *Saccharopolyspora erythraea*: HYDROXYLATION AT C-27.

MARVIN SCHULMAN, PATRICK DOHERTY, DEBORAH ZINK and BYRON ARISON

Merck Research Laboratories,
P. O. Box 2000, Rahway NJ 07065

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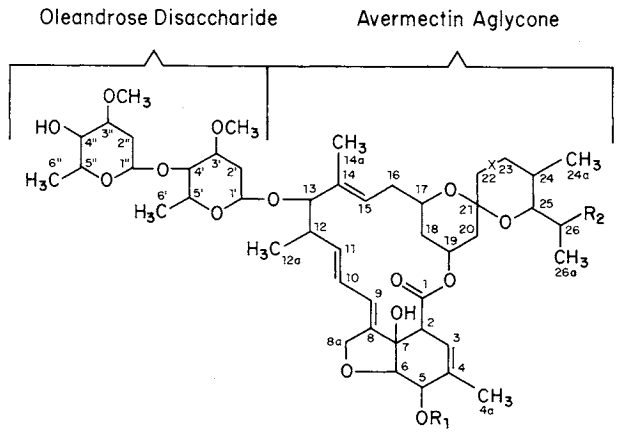
The avermectins, a family of secondary metabolites produced by *Streptomyces avermitilis*, are oleandrose disaccharide derivatives of sixteen membered macrolides with potent anthelmintic and insecticidal activity[1~4]. The general structure of the avermectins and of ivermectin, the synthetically derived 22, 23-dihydro derivative of avermectin $B_{1a}$ are shown in Fig. 1.

Avermectin derivatives in which the oleandrose disaccharide has been cleaved from the macrolide ring are termed aglycones. In earlier work described by SCHULMAN *et al*, *Saccharopolyspora erythraea* ATCC11635 was shown to hydroxylate ivermectin aglycone to the 28-hydroxy derivative[5]. This paper extends these studies and reports on the bioconversion of ivermectin aglycone to 2 isomers of the 27-hydroxy derivative by *S. erythraea* as shown in Fig. 2.

Frozen vegetative mycelia (FVM) of *S. erythraea* ATCC11635 were prepared by inoculating 250 ml of medium M102[6] in baffled 2-liter Erlenmeyer flasks with a lyophilized culture. The flasks were incubated at 32°C, 200 rpm and 85% relative humidity for 48 hours in a Kuhner cabinet. The packed cell volumes of the cultures were 8~12% and the pH ranged from 6.7~7.0. Aliquots (2 ml) of the cultures were frozen, stored at −80°C and used as a source of inoculum for seed cultures in future experiments. For seed cultures, 40 ml of medium M102[5] in 250-ml baffled Erlenmeyer flasks were inoculated with 1.0 ml of FVM and the flasks were incubated at 30°C, 85% relative humidity and 220 rpm for 40 hours.

Biotransformations were conducted in 250-ml Erlenmeyer flasks containing 40 ml of medium M102, a complex medium[6]. Each flask was inoculated with 1.0 ml of seed culture and the flasks were incubated at 30°C, 85% relative humidity and 220 rpm for 24 hours. At this time, 0.1 ml of dimethylsulfoxide containing 2.5 mg (4.24 μmol) of [$^3$H-22,23] 22,23-dihydro avermectin aglycone (specific activity = 1.5 × 108 dpm/μmol) was added and the flasks were incubated at 30°C, 85% relative humidity and 220 rpm for an additional 120 hours. Each flask was extracted twice with 80 ml of dichloromethane. The extracts were pooled, concentrated and chromatographed on Silica Gel-60 F254 precoated TLC plates (0.25 mm, E.M. Laboratories). The TLC plates were developed using dichloromethane-ethylacetate-methanol (9:9:1, v/v/v). The radioactive bands were located using a Bioscan 200 (Bioscan Inc.) and the individual bands were scraped from the plate and eluted with methanol. The

Fig. 1. General structure of the avermectins.




Avermectin terminology is as follows: $R_1$ = H in "B" components; $R_1$ = $CH_3$ in "A" components; x = CH=CH in "1" components; $R_2$ = $CH_2 \cdot CH_3$ in "a" components; $R_2$ = $CH_3$ in "b" components; x = CH·CHOH in "2" components; x = $CH_2 \cdot CH_2$ in ivermectins.

Fig. 2. Hydroxylation of ivermectin aglycone at C-27 by *S. erythraea*.

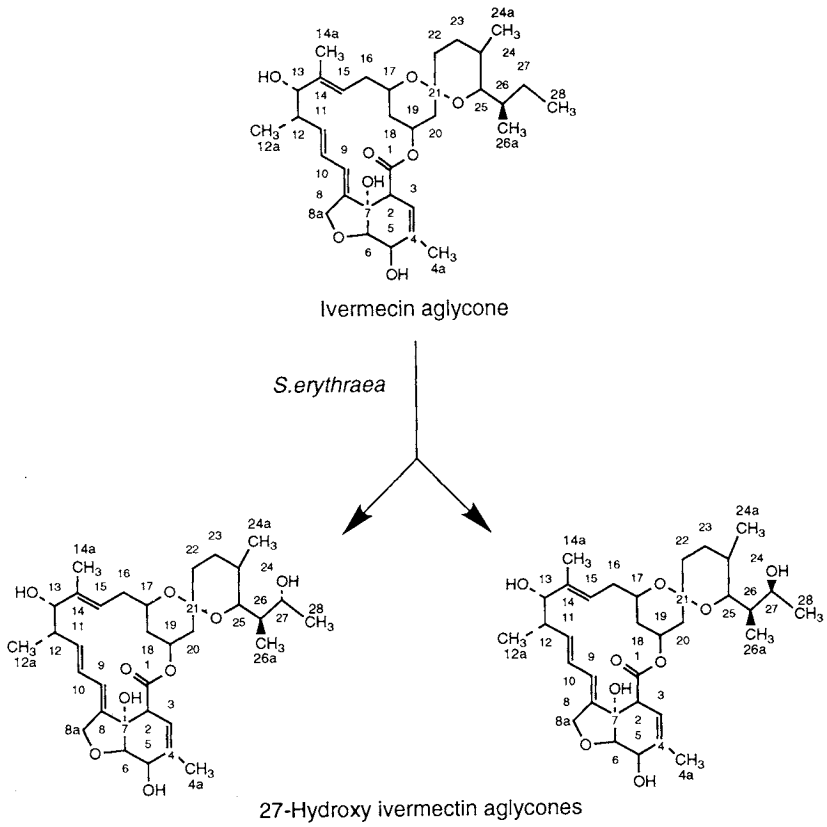
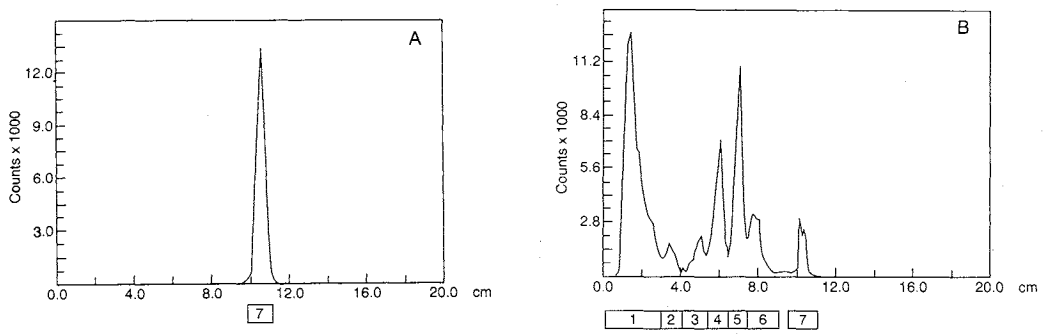


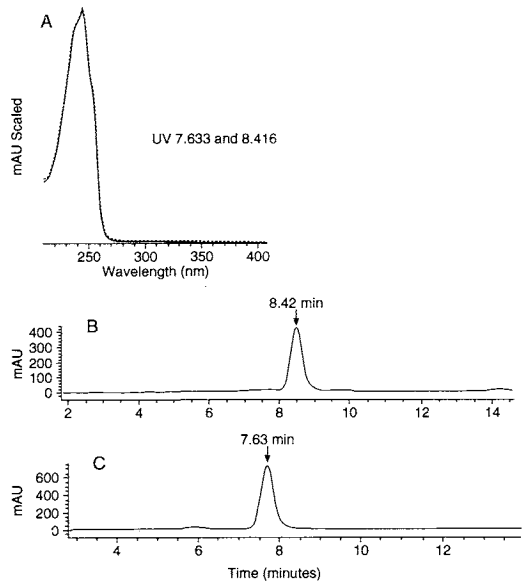

Fig. 3. TLC of the products of the biotransformation of ivermectin aglycone by *S. erythraea*.




Thin layer chromatography of the fed substrate and the extracts of the biotransformation was conducted as described above. Radioactivity in the products was measured with a Bioscan 200 for 10 minutes and the peaks were integrated. Panel A is the substrate, [$^3$H 22, 23] 22, 23-dihydro avermectin aglycone, panel B presents the biotransformation products.

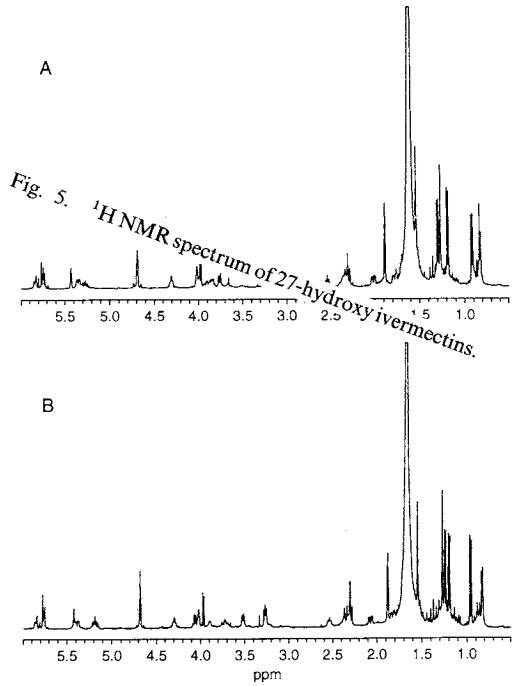
eluted bands were then further purified by HPLC on a Dupont Zorbax ODS reverse phase semi-prep column (9.4 mm × 25 cm) at 60°C with methanol-water (80 : 20) as the mobile phase at a flow rate of 3 ml/minute. The UV absorbtion spectra (200 ~ 400 nm) of the eluted peaks were monitored using a Hewlett Packard 1040a diode array spectrophotometer. Analytical HPLC (4.6 mm × 25 cm) was run on a Dupont Zorbax ODS reverse phase column at 60°C with methanol-water (80 : 20) as the mobile

Fig. 4. HPLC of 27-hydroxy ivermectin aglycones.




Approximately 10.0 $\mu$g of each isomer of purified 27-hydroxy ivermectin aglycone was chromatographed on Dupont Zorbax ODS column at 60°C with methanol-water (80:20) as the mobile phase and a flow rate of 1 ml/minute. Panel A is the diode array spectrum of the eluted peaks. (—) indicates the peak which elutes at 7.63 minutes, (------) denotes the spectra of the peak which elutes at 8.42 minutes; panel B is the elution profile of isomer B at 245 nm, panel C is the elution profile of isomer A at 245 nm.

Fig. 5. $^1$H NMR spectrum of 27-hydroxy ivermectins.




Purified samples of each isomer of 27-hydroxy ivermectin was dissolved in $CDCl_3$ and analyzed on a Varian XL-400 spectrometer. Panel A is isomer A; panel B is isomer B.

Table 1. Pertinent chemical shifts in the epimeric 27-hydroxy ivermectin aglycones.

| Derivative | 27-H | 28-$CH_3$ | 25-H | 17-H |
|---|---|---|---|---|
| Isomer A | 3.84 | 1.29 | 3.75 | 3.90 |
| Isomer B | 4.06 | 1.24 | 3.51 | 3.71 |

Spectra were measured in $CDCl_3$ at 400 MHz. Chemical shifts are expressed in ppm.

phase at 1 ml/minute. Structures were determined by mass and NMR spectroscopy. $^1$H NMR spectra were obtained in $CDCl_3$ at ambient temperature on a Varian UNITY 400 MHz spectrometer. Chemical shifts are in parts per million relative to internal tetramethylsilane. Mass spectra were recorded as previously described[7].

A Bioscan trace of the TLC plates is presented in Fig. 3. The substrate is shown in panel A and the extract of the biotransformation is shown in panel B. Following biotransformation, only a small amount of substrate (peak 7) remained and 6 new peaks appeared. Of these peaks, 3, 4, 5 and 6 were avermectins. Peak 3 (Rf 0.25) contained the 28-hydroxy ivermectin[5]. The two major peaks 4 (Rf 0.29) and 5 (Rf 0.33) contained the 27-hydroxy ivermectin aglycone isomers and peak 6 contained the 23-hydroxy derivative. A typical elution profile of the 27-hydroxy ivermectin aglycones from an analytical column and their UV absorption spectrum are shown in Fig. 4. Each isomer elutes as a single symmetrical peak and possesses a UV spectra typical of avermectins with an $E_{max}$ at 245 nm and a shoulder at 256 nm. The isomer with an elution time of 7.63 minutes was termed isomer A (TLC peak 5) and that eluting at 8.42 minutes was termed isomer B (TLC peak 4).

The $^1$H NMR spectra of isomers A and B of 27-hydroxy ivermectin are shown in Fig. 5. Key features in both spectra are the absence of the 28 methyl triplet, the presence of a novel fourth methyl doublet at 1.28 ppm and the downfield displaced 25-H signal. The latter is diagnostic of a nearby structural change and a chemical shift between 1.20~1.30 ppm for a methyl implies a functionality attached to the neighboring carbon. These in-

ferences were supported by a double irradiation experiment in which the 1.27 ppm methyl doublet collapsed to a singlet when a novel methine at 3.83 ppm (i.e., a region characteristic for HC–O) was irradiated. The assignment of these compounds as the 27-hydroxy derivatives was confirmed by mass analysis which indicated a molecular weight of 602.772 for each isomer. The chemical shifts of 25-H, 27-H, the 28-$CH_3$ and 17-H are diagnostic for distinguishing the 27-hydroxy epimers. These values are presented in Table 1. The absolute stereochemistry of the isomers has not been determined.

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