MICROBIAL CONVERSION OF AVERMECTINS BY *Saccharopolyspora erythrea*: HYDROXYLATION AT C28

MARVIN SCHULMAN, PATRICK DOHERTY, DEBORAH ZINK and BYRON ARISON

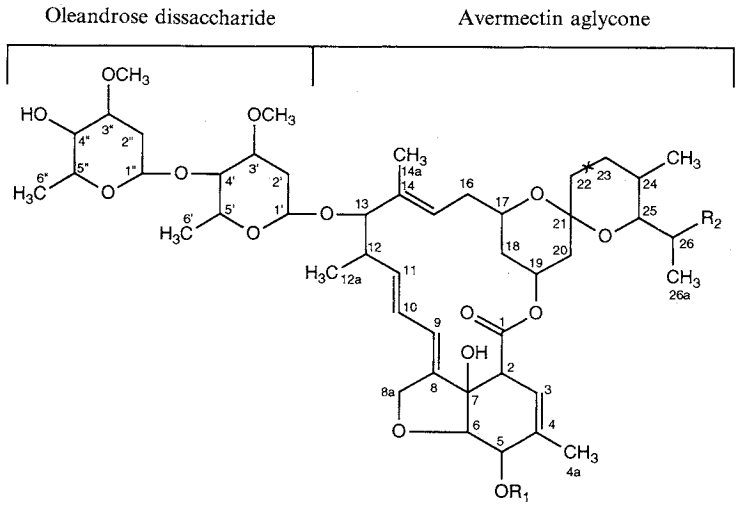
Merck Research Laboratories,
P.O. Box 2000, Rahway NJ 07065

(Received for publication January 13, 1993)

Avermectins are a family of oleandrose disaccharide derivatives of sixteen membered macrolides with potent anthelmintic and insecticidal activity produced by *Streptomyces avermitilis*[1~4]. The structure of ivermectin, the synthetically derived 23,23-dihydro derivative of avermectin B1a is shown in Fig. 1. Compounds in which the oleandrose has been cleaved are termed aglycones. This paper reports the bioconversion of ivermectin aglycone (22,23-dihydro avermectin B1a aglycone) to the 28-hydroxy derivative by *Saccharopolyspora erythrea* ATCC11635 as shown in Fig. 2.

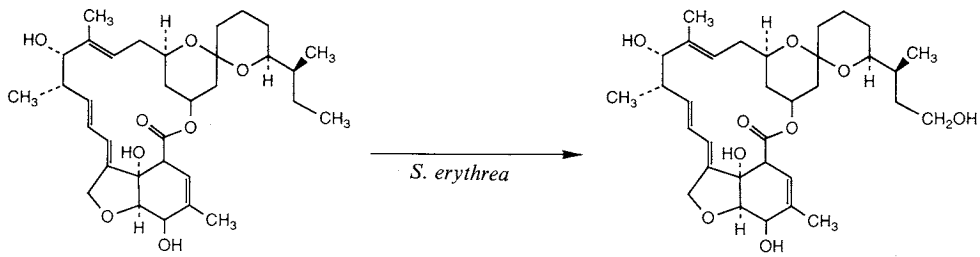
Frozen vegetative mycelia (FVM) of *S. erythrea* ATCC11635 were prepared by inoculating 250 ml of medium M102[5] in baffled 2-liter Erlenmeyer flasks with a lyophilized culture. The flasks were incubated at 32°C, 220 rpm and 85% relative humidity for 48 hours in a Kuhner cabinet. The packed cell volumes of the cultures were 8~12% and the pH ranged from 6.7 to 7.0. Aliquots (2 ml) of the culture 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

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_2CH_3 in "a" components; R_2 = CH_3 in "b" components; x = CHCHOH in "2" components; x = CH_2CH_2 in invermectins.

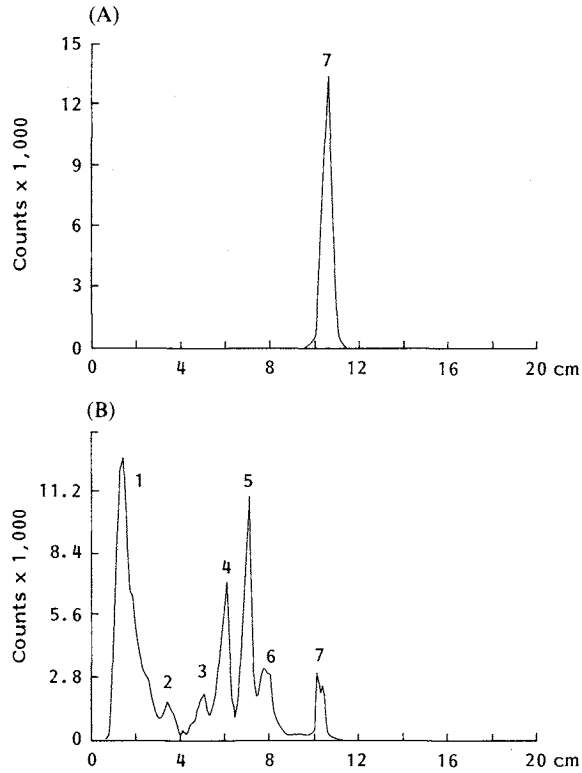
Fig. 2. Hydroxylation of ivermectin aglycone by *S. erythrea*.



Ivermectin aglycone

28-Hydroxy ivermectin aglycone

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



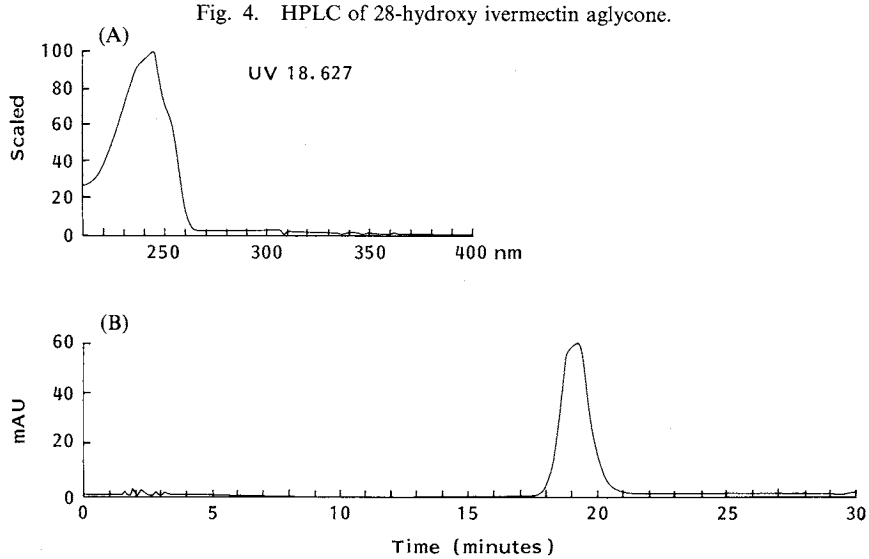
Thin layer chromatography of the fed substrate and the extracts of the biotransformation was conducted as described above. Radioactivity in the products was measured with a Bioscan 200 for 10 minutes and the peaks were integrated. Panel A is the substrate, [^{3}H-22,23]22,23-dihydro avermectin aglycone,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

M102[5] in 250 baffled Erlenmeyer flasks were inoculated with 1.0 ml of FVM and the flasks were incubated at 30°C, 85% relative humidity and 200 rpm for 40 hours.

The biotransformations were conducted in 250-ml Erlenmeyer flasks containing 40 ml of medium M102[5]. 1.0 ml of seed culture was added to each flask as inoculum 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 (2.5 mg, specific activity = 1.5×10^8 dpm/μmol) was added and the flasks were incubated at 30°C, 85% relative humidity and 220 rpm for 120 hours. Each flask was extracted twice with 80 ml portions of dichloromethane. The extracts were pooled, concentrated and chromatographed on Silica Gel-60 F_{254} precoated TLC plates (0.25 mm, E. M. Laboratories). The TLC plates were developed using dichloromethane-ethylacetate-methanol (9:9:1). 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 eluted bands were then further purified by HPLC on a Dupont Zorbax ODS reverse phase semi-prep column (9.4 mm i.d. × 25 cm) at 60°C with methanol-water (80:20) as the mobile phase at a flow rate of 1 ml/minute. The UV absorbtion spectra (200~400 nm) of the eluted peaks were monitored using a Hewlett Packard 1040a diode array spectrophotometer. Structures were determined by mass spectroscopy 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[6].

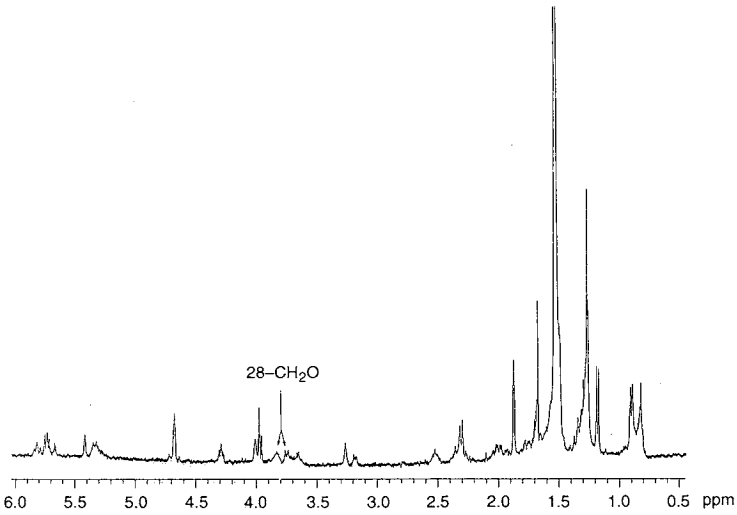
Fig. 3 presents a Bioscan trace of the TLC plates. The substrate fed is shown in panel A and the extract of the biotransformation is shown in panel B. Follwoing biotransformation, only a small amount

Fig. 4. HPLC of 28-hydroxy ivermectin aglycone.



Approximately 2.0 μg of purified 28-hydroxy ivermectin 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 peak; panel B is the elution profile at 245 nm.

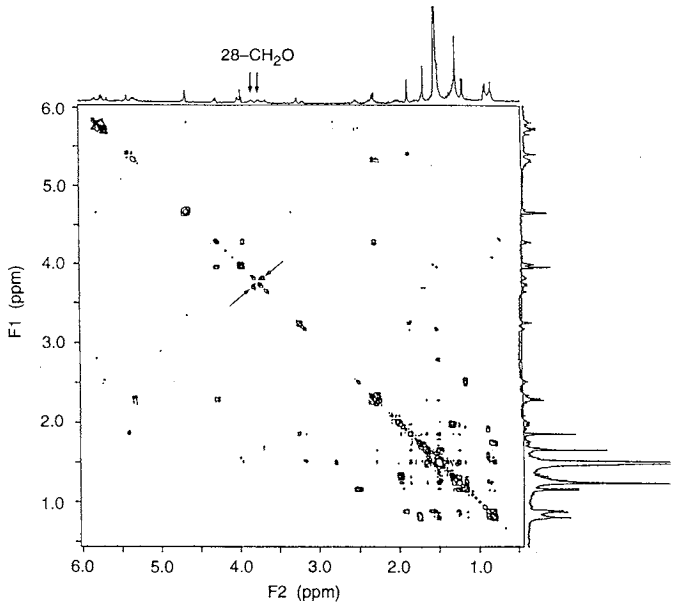
Fig. 5. ^{1}H NMR spectrum of 28-hydroxy ivermectin.



A purified sample of 28-hydroxy ivermectin was dissolved in $CDCl_3$ and analyzed on a Varian UNITY 400 MHz spectrometer.

of the substrate (peak 1) 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 (Peaks 4, 5, and 6 contained other hydroxylated avermectins which are the subject of another manuscript.). A typical elution profile from an analytical column (4.6 mm × 25 cm) of the 28-hydroxy ivermectin and its UV absorbtion spectrum are shown in Fig. 4. The compound elutes as a single symmetrical peak and possess a UV spectra typical of avermectins with an E_{max} at 245 nm and a shoulder at 256 nm. The molecular weight of the 28-hydroxy derivative was determined by mass analysis to be 602.772. The ^{1}H NMR spectrum

Fig. 6. COSY ^{1}H NMR spectrum of 28-hydroxy ivermectin.



The arrows indicate cross peaks of the 28-CH_2O, which are diagnostic of coupling between these novel protons.

of the 28-hydroxy ivermectin is shown in Fig. 5. Key features of this spectrum are the absence of a 28-methyl triplet at 0.94 ppm and the presence of two novel protons in the region characteristic of CH attached to oxygen (3.8~3.9 ppm). Although a CH_2O grouping is a reasonable inference from these observations and the mass spectral evidence that an oxygen has been incorporated, it was deemed important to strengthen this position by establishing that these protons were coupled to each other and hence in close proximity. This was demonstrated in the COSY spectrum (Fig. 6) which clearly shows cross peaks diagnostic of coupling between the novel protons.

References

1) BURG, R. W.; B. M. MILLER, E. E. BAKER, J. BIRNBAUM, S. A. CURRIE, R. HARTMAN, Y. KONG, R. L. MONAGHAN, G. OLSON, I. PUTTER, J. B. TUNAC, H. WALLICK, E. O. WALLICK, R. OIWA & S. OMURA: Avermectins, new family of potent anthelmintic agents: producing organism and fermentation. Antimicrob. Agents Chemother. 15: 351~367, 1979

2) EGERTON, J. R.; D. A. OSTLIND, L. S. BLAIR, E. H. EARY, S. SUHAYDA, R. F. CEFELLI & W. C. CAMPBELL: Avermectins, new family of potent anthelmintic agents. Efficacy of the B1a component. Antimicrob. Agents Chemother. 15: 372~378, 1979

3) MILLER, T.; L. CHAIT, D. J. COLE, L. J. COLE, J. E. FLOR, R. T. GOEGELMAN, V. P. GULLO, H. JOSHUA, A. J. KEMPF, W. R. KRELLOWITZ, R. L. MONAGHAN, R. E. ORMAND, K. E. WILSON, G. ALBERS-SCHONBERG & I. PUTTER: Avermectins, new family of potent anthelmintic agents: Isolation and chromatographic properties. Antimicrob. Agents Chemother. 15: 368~371, 1979

4) OSTLIND, D. A.; S. CEFELLI & R. LANG: Insecticidal activity of the anti-parasitic avermectins. Vet. Rec. 105: 168, 1979

5) CORCORAN, J. W.: S-Adenosylmethionine: erythromycin C *O*-Methyltransferase. Methods In Enzymology 43: 487~498, 1975

6) SCHULMAN, M. D.; D. L. VALENTINO, O. D. HENSENS, D. ZINK, M. NALLIN, L. KAPLAN & D. A. OSTLIND: Demethylavermectins. Biosynthesis, isolation and characterization. J. Antibiotics 38: 1494~1498, 1985