Maduramicin α: Characterization of ^{14}C-Derived Residues in Turkey Excreta

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Maduramicin α, a highly potent polyether ionophore antibiotic for preventing coccidiosis in poultry, is passed predominantly in turkey excreta following oral feeding. Following isolation and purification, the turkey excreta metabolites were characterized primarily by liquid chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry. Maduramicin α and its metabolites generate a characteristic pair of ions corresponding to $(M + NH_4)^+$ and $(M + Na)^+$ which assist in differentiating the metabolites from matrix coextractives. These two ions also fragment differently in tandem mass spectrometry, thus providing structural information for characterizing the nature of unknown metabolites. The primary metabolic pathway of maduramicin α in the turkey is O-demethylation at one or more of the methoxy groups. Hydroxylation and glucuronide conjugation are minor secondary metabolic processes.

INTRODUCTION

Maduramicin α (Figure 1), a polyether carboxylic ionophore antibiotic produced by the bacterium *Actinomadura yumaensis*, is highly effective for the prevention of coccidiosis in poultry (Liu et al., 1983). Previous investigations on the metabolism of maduramicin α in the chicken and rat have shown the major liver metabolites to result from O-demethylation of the parent (Brown and Rajan, 1986). The only difference between the two species was the site of O-demethylation. The chicken liver metabolite lost the methyl group at R_{47} (Figure 1) of the A-ring, generating maduramicin β, a known minor product in the production of maduramicin α. The rat liver metabolite showed loss of the R_{45} methyl from the G-ring. Since the maduramicin α is extensively excreted in turkey excreta during oral dosing, the objective of this study was to isolate major metabolites from the turkey excreta and to characterize their chemical structures.

MATERIALS AND METHODS

^{14}C-Radiolabeled Maduramicin α. The ammonium salt of maduramicin α labeled with carbon-14 at seven different positions was obtained from American Cyanamid Co., Agricultural Research Division (Princeton, NJ). Preparation of the test compound was accomplished by incorporating carbon-14 propionate in the fermentation medium. The structure of the ^{14}C-labeled maduramicin α and the location of the ^{14}C-labeled atoms are shown in Figure 1 and were deduced from a previous ^{13}C NMR study of ^{13}C-enriched maduramicin α (Tsou et al., 1984). The sodium salts of unlabeled maduramicin α and unlabeled maduramicin β were also available from American Cyanamid Co. for use as chromatographic standards.

Dosing Turkeys. A total of 901 mg of ^{14}C-labeled maduramicin α having a specific activity of 1.26 μCi/mg was dissolved in 4.5 g of benzyl alcohol, transferred onto 84.5 g of Grit-O-Cobs granules (40/60 mesh, The Andersons, Maumee, OH), and mixed in a tumbler for 1 h; 53.79 g of the premix was blended with 2 kg of Cyanamid Turkey Grower Diet 668 (Lot 000084) in a Hobart blender for 10 min. This preblend was combined with 73 kg of the same grower diet in a stainless steel barrel and mixed by end-to-end rotation for 30 min. Liquid scintillation spectrometry of acetonitrile extracts of the diet indicated a level of 7.3 ppm of maduramicin α at a specific activity of 1.26 μCi/mg (2797 dpm/μg).

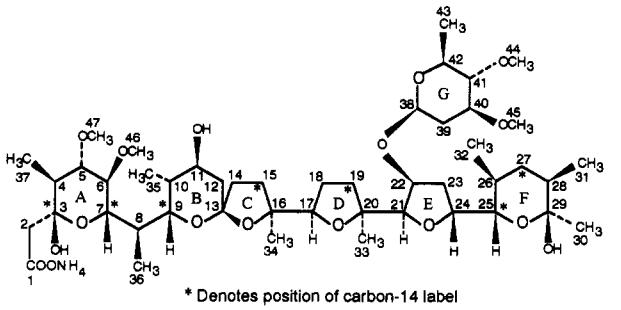

Figure 1. Structure of the ammonium salt of maduramicin α.

Thirty Nicholas Broad White turkeys (15 males and 15 females) approximately 10 weeks of age with an average weight of 4.2 kg (range 3.2–5.2 kg) were offered ad libitum medicated feed for 7 consecutive days. Five control turkeys (three males and two females) were fed nonmedicated feed for the same time period. The turkey excreta was collected daily in plastic trays lined with plastic sheets placed under the wire flooring of the cages. Excreta of each individual animal were collected separately and kept frozen until the time of analysis. The collection spanned the 7 days of treatment and 3 days of withdrawal (Wong, 1988).

Excreta Analysis: Sample Combustion and Radioanalysis. The excreta samples were individually pulverized in frozen state in dry ice. Two methods of radioanalysis were used interchangably. Half-gram homogenized samples were weighed directly into combustion cones and combusted. Alternatively, 5 g of the samples was homogenized with 5 mL of deionized water by using a Brinkmann Polytron homogenizer (Brinkmann Instruments, Inc. Westbury, NY). A 1.0-g sample of homogenate equivalent to 0.5 g of excreta was placed onto an absorbant combustion pad and combusted in a Packard Tricarb Model 306 sample oxidizer (Packard, Sterlings, VA). Triplicate samples were combusted. If the triplicates showed greater than 10% variation from the mean, those samples were repeated. The $^{14}CO_2$ released by the combustion was trapped in a scintillation vial containing 9 mL of Oxisorb 2 and 12 mL of Oxiprep 2 (New England Nuclear Co.). The radioactivity was measured by using a Beckman LS5801 scintillation spectrometer (Beckman Instruments, Fullerton, CA). Samples producing counts less than twice background were considered below the level of radioanalysis

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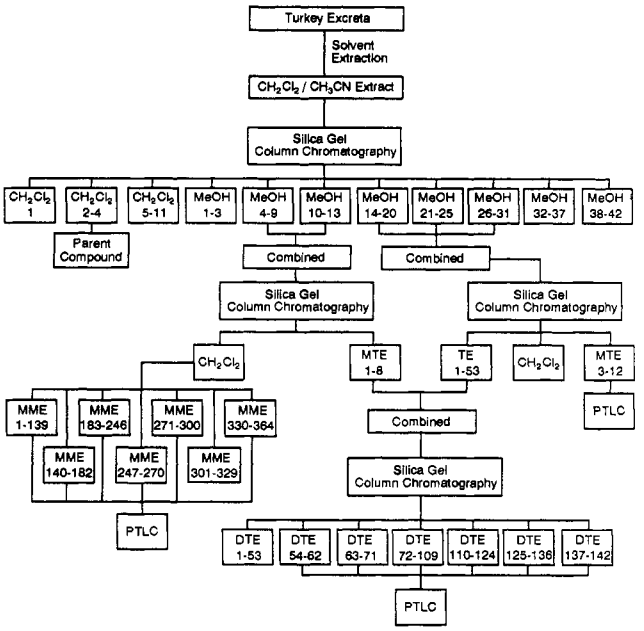

Figure 2. Flow chart for isolation of metabolites from turkey excreta.

sensitivity. Using a specific activity of 1.26 μCi/mg and a sample of weight of 0.5 g, control excreta samples were validated to detect 0.059 ppm (Wong, 1988).

Sample Extraction and Fractionation. For the characterization of metabolite structures, excreta samples from day 5 were pooled (estimated 27.6 mg of maduramicin α equivalent residues) and processed in 100–200-g batches. Each batch was extracted three times with 3× volume of acetonitrile (ACN). The ACN extracts were filtered, pooled, and concentrated in a rotary evaporator to remove ACN. The remaining residue (mainly as aqueous phase) was partitioned with methylene chloride (CH_2Cl_2, v/v × 3) to yield an organosoluble fraction and an aqueous soluble fraction. The solid after ACN blending was designated a post-extraction solid (PES). The CH_2Cl_2 fraction was concentrated in a rotary evaporator and used for isolation and characterization work.

Enzyme Hydrolysis of Aqueous Fraction. The aqueous fraction (800 000 dpm used) was added to sodium acetate (1.014 g) and glacial acetic acid (0.15 mL) to form acetate buffer at pH ~5.5 and then was added 150 mg of β-D-glucuronidase (Sigma Chemical Co.) for incubation at 37 °C in a constant-temperature water bath with shaking. An additional 75 mg of enzyme was added to the incubation mixture 4 h after the incubation was initiated. The incubation procedure continued for a total of 24 h. The sample was cooled to room temperature and partitioned with CH_2Cl_2 (v/v × 2) to yield an enzyme-released aglycon fraction and polar aqueous soluble fraction. The CH_2Cl_2 extract was concentrated under reduced pressure and analyzed by TLC.

Thin-Layer Chromatography (TLC). Metabolite fractions were analyzed by TLC and autoradiography. Designated fractions and metabolites were chromatographed on silica gel TLC (E. Merck or J. T. Baker, silica gel G, 250 μm) and developed with the following solvent systems: hexane/acetone (1:1); toluene/ethyl acetate/methanol (70:100:30); ethyl acetate/ether/ethanol/triethylamine/H_2O (100:100:3:4:3.5). Developed TLC plates were monitored by a TLC radioactivity image scanner (AMBIS System). Visualization of TLC spots for reference compounds and/or metabolites was accomplished by spraying the plate with a methanolic solution containing 4 g of vanillin (Aldrich Chemical Co.) and 4 mL of concentrated sulfuric acid in 100 mL of methanol, followed by heating in an oven at 100 °C for 10 min. A bluish purple color is indicative of the presence of maduramicin-related compounds.

Preparative Isolation and Purification of Metabolites. The radiolabeled metabolites in the concentrated crude CH_2Cl_2 fraction were isolated and purified for structural characterization following the scheme shown in Figure 2. A silica gel column (J. T. Baker, 40 μm, 30 g) was eluted with CH_2Cl_2 followed by an increasing percentage (1–12%) of MeOH in CH_2Cl_2. Fractions of 50–100 mL were collected. Eight major fractions containing radioactivity were regrouped into four fractions on the basis of similarities of TLC R_f values. These four fractions were CH_2Cl_2, MeOH (4–13), MeOH (14–31), and MeOH (32–42).

Additional fractionation of MeOH (14–31) was performed after it was concentrated to dryness, redissolved in CH_2Cl_2, and loaded onto a 30-g silica gel column (1 in. × 12 in. × 35–70 mesh). The column was eluted with 200 mL of CH_2Cl_2 followed by 1240 mL of toluene/EtOAc (70:100) and MeOH/toluene/EtOAc [15:70:100 (325 mL) and 20:70:100 (370 mL)], generating fractions TE (1–53) and MTE (1–12).

MeOH (4–13) was further fractionated by loading onto a silica gel column (30 g, 40 μm) and eluting with 450 mL of CH_2Cl_2 followed by MeOH/toluene/EtOAc [15:70:100 (250 mL) and 30:70:100 (485 mL)]. The CH_2Cl_2 fractions were pooled, loaded onto a 30-g silica gel column (40 μm), and eluted with CH_2Cl_2 followed by an increasing percentage of MeOH (0.5%, 1%, 2%, 4%, 8%, and 15%) in CH_2Cl_2. A total of 364 fractions (MME 1–364) were collected and pooled according to their R_f values on TLC. The MTE (1–8) fractions from MeOH (4–13) were pooled with TE (1–53) from MeOH (14–31) and fractionated again by silica gel column chromatography using CH_2Cl_2/toluene/EtOAc, and MeOH/toluene/EtOAc as described for MeOH (14–31) to give fractions DTE 1–142. Fractions thus obtained were analyzed together on one TLC plate to combine metabolites having the same R_f values. Appropriate fractions were then pooled and combined for additional purification by preparative TLC (PTLC).

The concentrated samples from open column chromatographic separation were streaked on TLC plates (EM Science, silica gel, 500 μm, 20 cm × 20 cm) as a band and developed with hexane/acetone (1:1) to a distance of 15 cm from origin, three times. The plates were air-dried at room temperature in the hood and scanned by AMBIS image scanner to locate the radioactive bands. Each radioactive band was scraped off the plate and eluted with CH_2Cl_2/MeOH (1:1). The eluted radioactive samples designated PTLC fractions were spotted separately on analytical TLC plates and developed with the same solvent system to locate the metabolite of interest according to their R_f value. The radioactivity in each PTLC fraction was determined by counting an aliquot from each fraction. Fractions were also concentrated by evaporation and analyzed by silica gel TLC to determine radiopurity. Repetitive PTLC was performed if the isolated sample was not homogeneous.

The metabolite fractions purified by the procedures described above were further purified by reverse-phase PTLC using TLC plate coated with C-18 absorbant (J. T. Baker, 200 μm, 20 cm × 20 cm). The sample was streaked as a band and developed once with MeOH/H_2O (80:20) to a distance of 15 cm from origin. The radioactive bands were located by image scanner, scraped off, and eluted with CH_2Cl_2/MeOH (1:1). The radioactivity in each fraction was determined by counting an aliquot from each fraction. Fractions were also evaporated and spotted on silica gel TLC plates for purity check. The metabolites obtained from this procedure were then subjected to mass spectrometric characterization.

Thermospray Liquid Chromatography/Mass Spectrometry (TSP LC/MS). The purified metabolites were analyzed by TSP LC/MS using conditions essentially identical with those reported previously from the confirmatory analysis of maduramicin α in chicken fat (Stout et al., 1989). The same CH_3OH/H_2O (90/10, 0.1 N ammonium acetate) mobile phase was employed. The only differences were the uses of a Finnigan-MAT TSQ-70 triple-stage quadrupole system and an ABI Kratos Spectroflow Model 400 liquid chromatograph in the present study.

Thermospray Liquid Chromatography/Tandem Mass Spectrometry (TSP LC/MS/MS). The TSP LC/MS/MS experiments utilized the same instrumentation, LC conditions, and LC/MS interface parameters as those detailed for the TSP LC/MS experiments. Additional MS/MS parameters included the following: collision gas and pressure, Ar at 1.0 mT; collision energy, −5 or −10 eV; resolution of the parent ion, 2 u at half-height. For the neutral loss experiments, the first and third qua-

drupoles were scanned together with the third quadrupole lagging the first quadrupole by 62 u. For the daughter experiments, the first quadrupole was set on the parent ion of the metabolite while the third quadrupole scanned the daughter ions.

High-Performance Liquid Chromatography (HPLC). Final purification of metabolite G for NMR analysis required HPLC using a Waters Powerline HPLC system. A Du Pont Zorbax ODS column (4.6 mm × 25 cm) with a methanol/water (90/10) mobile phase flowing at 1.0 mL/min gave a retention time of approximately 4 min. The metabolite was monitored by UV absorbance at 214 nm and by collecting fractions for liquid scintillation counting.

Nuclear Magnetic Resonance (NMR). Metabolite G was analyzed by both proton and carbon-13 NMR techniques. The analysis was performed at Spectral Data Services, Inc., 818 Pioneer, Champaign, IL 61820, by using a reconstructed 360-MHz instrument. The spectra were obtained in $CDCl_3$ solution. Proton NMR was performed in the 0–14 ppm range and carbon-13 NMR was performed in the 0–230 ppm range.

Total ^{14}C Residue Analysis. Levels of radioactivity in post-extraction solids (PES) were determined by combustion of ~0.2-g subsamples in a Harvey OX-300 biological sample oxidizer. All combustions were performed in triplicate or duplicate. Statistical correction for instrument recovery was performed by using [^{14}C]mannitol as a standard.

Liquid Scintillation Counting (LSC). Radioactivity in various extracts and TLC scrapings was determined by counting in 5 mL of Ready Value (Beckman) or 5 mL of Hydrocount (J. T. Baker). All samples were routinely counted in a Beckman LS 3801 or 5000 TD liquid scintillation counter. Counting time was to a statistical accuracy of ±2% or a maximum of 10 min, whichever came first. Shorter counting times (1–2 min) were used in certain cases due to a range-finding experiment for column separation conditions. The Beckman LS 3801 and 5000 TD were programmed to automatically subtract background and convert cpm to dpm.

RESULTS AND DISCUSSION

Isolation and Purification of Metabolites. The excretion of carbon-14 material averaged 49.0% (range 32.6–68.5%) in the first day and 68.1% (range 50.8–85.7%) after day 3 of withdrawal (Wong, 1988). The sample extraction and fractionation of the pooled day-5 samples gave a distribution of radioactivity in CH_2Cl_2, aqueous, and PES of 82.4%, 7.1%, and 9.5%, respectively. Following the isolation and purification scheme shown in Figure 2, nine metabolites in addition to the parent were separated. The percent distribution of the metabolties in turkey excreta were A (parent, 23.8%), B (1.2%), C (4.2%), D2 (3.3%), E (0.6%), F2b (3.3%), G (17.9%), H (3.3%), and I-1 and I-3 (5.3%); 12.6% of the radioactivity was polar, origin-bound material. The 2-D TLC profile of the turkey metabolites is shown in Figure 3. This silica gel plate (250 μm) was developed three times in the first dimension with hexane/acetone (1:1) and once in the second dimension with MeOH/toluene/EtOAc (3:7:10).

TSP LC/MS Characterization of Metabolites. TSP LC/MS has been shown to be a highly effective analytical technique both for separating maduramicin α from matrix coextractives prior to MS analysis and for generating molecular weight information by MS (Stout et al., 1989). Maduramicin α generated a characteristic pair of ions at $(M + NH_4)^+$ and $(M + Na)^+$ which served to differentiate the analyte from matrix coextractives. These two ions in the same ratio were generated regardless of whether the ammonium salt or sodium salt of maduramicin α was analyzed and probably resulted from the well-known proclivity of polyether ionophores for traces of sodium (Occolowitz and Hamill, 1983; Tabet et al., 1985) combined with NH_4^+ as the ionizing species. Since all the turkey excreta metabolites were more polar than the parent, use of reverse-phase LC with previously reported conditions

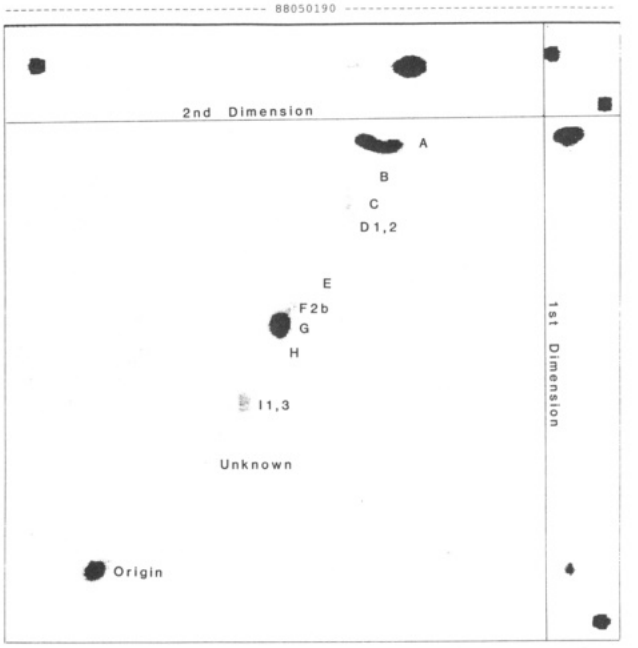

Figure 3. Two-dimensional TLC profile of the turkey metabolites.

Table I. Molecular Weight Information Expressed as Nominal Mass Values from TSP LC/MS

metabolite	MW	$(M + NH_4)^+$	$(M + Na)^+$	comment
maduramicin α	916	934	939	parent compound
maduramicin β	902	920	925	monodemethylated
C	902	920	925	monodemethylated
D2	902	920	925	monodemethylated
E (major)[a]	932	950	955	monohydroxylated
E (minor)	902	920	925	monodemethylated
F2b	888	906	911	didemethylated
G	888	906	911	didemethylated
H	918	936	941	monohydroxylated and monodemethylated
I-1	874	892	897	tridemethylated
I-3 (major)	918	936	941	monohydroxylated and monodemethylated
I-3 (minor)	904	922	927	monohydroxylated and didemethylated

[a] Indicates the major component in an isolate showing multiple components by mass spectral analysis.

(Stout et al., 1989) guaranteed that the metabolites would elute before the parent. The results of the TSP LC/MS analyses of the metabolites are summarized in Table I. The molecular weight differences of the metabolites from the parent fall into three categories: (1) losses of multiples of 14 u indicating demethylation; (2) additions of 16 u indicating hydroxylation; and (3) combinations of (1) and (2).

TSP LC/MS/MS Characterization of Metabolites. Tandem mass spectrometry (MS/MS) has demonstrated itself to be a powerful technique for structural characterization of unknown organic compounds (Perchalski et al., 1982; Coutant et al., 1987) and has been especially useful when used in conjunction with a "soft" MS ionization technique, such as TSP LC/MS, which induces little initial fragmentation (Covey et al., 1986; Voyskner et al., 1987). High-energy collisionally activated dissociation (CAD) spectra have been reported for the $(M + NH_4)^+$ and $(M + Na)^+$ ions of maduramicin α and maduramicin β (Siegel et al., 1987) and agreed well with the low-energy CAD spectra used in the TSP LC/MS/MS confirmatory method for maduramicin α (Stout et al., 1989). The $(M + NH_4)^+$

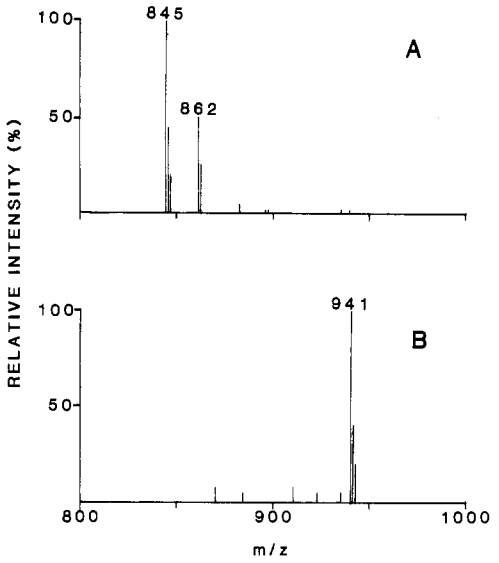

Figure 4. Mass spectra of metabolite I-3 from (A) TSP LC/MS analysis and (B) TSP LC/MS/MS analysis with neutral loss of 62 u.

and $(M + Na)^+$ ions of the reference compounds gave radically different CAD spectra, thus offering two different venues for structural characterization of the metabolites.

Under MS/MS conditions, the $(M + Na)^+$ ions of the reference compounds gave almost solely a concerted loss of $CO_2 + H_2O$ (62 u) as long as the carboxylic acid group and the hemiketal hydroxyl group were retained on the A-ring. This fragmentation pathway allowed screening of isolates for metabolites by detecting only parent ions that lost 62 u following CAD. While the major impact of these neutral loss MS/MS experiments was to confirm the molecular weight assignments from the TSP LC/MS analyses, in several instances the greatly enhanced specificity of MS/MS permitted the detection of metabolites that were obscured by intense ions arising from coeluting matrix coextractives. The TSP LC/MS/MS analysis of metabolite I-3 using a neutral loss of 62 u highlights the utility of the MS/MS experiment for locating a metabolite obscured by a complex matrix. As shown in Figure 4, the major ions in the TSP LC/MS spectrum are m/z 845 and 862. Neither ion shows a second ion 5 u away indicative of a maduramicin α metabolite. The TSP LC/MS/MS analysis (neutral loss of 62 u) of this isolate reveals the presence of only the parent ion at m/z 941. This ion corresponds to the $(M + Na)^+$ ion of a monohydroxylated–monodemethylated metabolite and represents less than 1% of the response of the coeluting interference in the original TSP LC/MS analysis. The ions at m/z 845 and 862 in the TSP LC/MS analysis are no longer detected because they fail to lose 62 u when undergoing collision in the MS/MS experiment.

Under MS/MS conditions, the $(M + NH_4)^+$ ions of maduramicin α and maduramicin β fragmented much more extensively than the $(M + Na)^+$ ions and generated several structurally significant ions for the characterization of unknown metabolites. These ions included m/z 629 and 647, which characterize the backbone of the parent (rings B-C-D-E-F), and fragments a and g, which retain the A-ring and G-ring, respectively (Siegel et al., 1987). Thus, by selecting the $(M + NH_4)^+$ ion of the metabolite in the first quadrupole, fragmenting the $(M + NH_4)^+$ ion by CAD in the second quadrupole, and scanning the resultant fragment ions with the third quadrupole, a "daughter ion" spectrum characterizing the metabolite was generated. As shown in Figure 5, both the maduramicin α and maduramicin β standards and the major didemethylated metabolite G generate ions at m/z 629 and 647, indicating the backbone of the parent is present in all three. Fragment a at m/z 741 in the parent is shifted 14 u lower in maduramicin β to m/z 727, indicating loss of a methyl group from the A-ring. In the didemethylated metabolite G, the m/z 741 ion indicates retention of both methyl groups on the A-ring and, consequently, loss of both methyl groups from the G-ring. This deduction is supported by the m/z 523 ion (fragment g), which is 28 u lower than fragment g (m/z 551) from the parent. In an identical manner, the daughter spectra generated by TSP LC/MS/MS were used to locate the sites of O-demethylation in the O-demethylated metabolites. Table II summarizes the results of these investigations and shows the retention of the m/z 629 and 647 ions indicating the unaltered backbone of the parent in the O-demethylated metabolites. Metabolite D2 generated daughter ion spectrum nearly identical with that of maduramicin β and was, therefore, tentatively assigned that structure. The daughter ion spectra of the hydroxylated metabolites are radically different from the O-demethylated metabolites. As shown in Figure 5 for the hydroxylated metabolite E, most of the fragmentation is concentrated above m/z 800 and corresponds to simple losses of combinations NH_3, H_2O, and CO_2. The daughter ions in the mass region of fragment a, fragment g, m/z 629, and m/z 647 did not correlate with the reference compounds, the O-demethylated metabolites, or even among the hydroxylated metabolites themselves. Consequently, no information could be gleaned from the TSP LC/MS/MS experiments regarding the sites of hydroxylation in the hydroxylated metabolites.

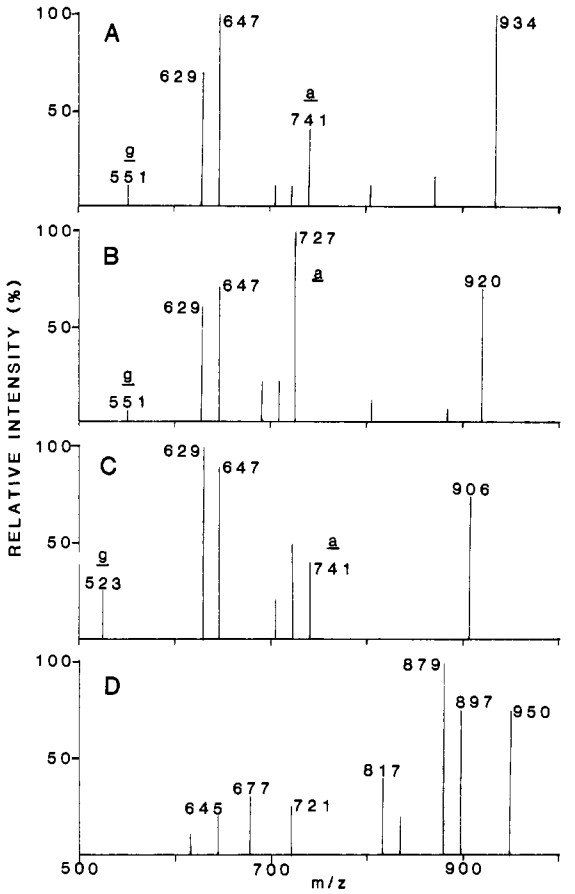

Figure 5. Daughter ion spectra from TSP LC/MS/MS of the $(M + NH_4)^+$ ions of (A) parent, (B) maduramicin β, (C) metabolite G, and (D) metabolite E.

NMR Confirmation of Metabolite G. HPLC-purified

Table II. Nominal Mass Values from TSP LC/MS/MS for Neutral Loss of 62 u and Daughter Ion Scan

		daughter ion scan of (M + NH_4)					
metabolite	loss of 62	(M + NH_4)	fragment a	fragment g	backbone	others	comment
maduramicin α	939	934	741 (40)[a]	551 (10)	647 (100) 629 (70)		parent compound
maduramicin β	925	920	727 (100)	551 (5)	647 (70) 629 (60)		mono-O-demethylated at C-47
C	925	920	741 (40)	537 (15)	647 (60) 629 (100)		mono-O-demethylated at G-ring
D2	925	920	727 (100)	551 (10)	647 (60) 629 (45)		mono-O-demethylated at C-47
E (major)[b]	955	950				897 (75), 879 (100), 836 (30), 817 (40), 721 (25), 677 (25), 645 (30)	monohydroxylated of parent
F2b	911	906	727 (100)	537 (5)	647 (100) 629 (65)		O,O-didemethylated, one from A-ring and one from G-ring
G	911	906	741 (30)	523 (20)	647 (90) 629 (100)		O,O-didemethylated at C-44, C-45
H	941	936				883 (60), 865 (50), 803 (35), 659 (20), 645 (30)	monohydroxylated and mono-O-demethylated
I-1	897	892	727 (100)	523 (100)	647 (85) 629 (55)		O,O,O-tridemethylated at C-44, C-45, and one from A-ring
I-3 (major)	941	936				883 (80), 865 (70), 645 (30)	monohydroxylated and monodemethylated

[a] Number in the parentheses is the percent of ion intensity. [b] Indicates the major component in an isolate showing multiple components by mass spectral analysis.

metabolite G (650 μg) was analyzed by proton and carbon-13 NMR. The two higher field methoxy singlets in the proton NMR spectrum of the parent are absent in metabolite G. In the parent, these two resonances arise from the two methoxy groups on the G-ring (Brown and Rajan, 1986). Their absences in the proton NMR of metabolite G confirm the results from TSP LC/MS/MS that metabolite G is di-O-demethylated on the G-ring. Attempts to obtain a carbon-13 NMR spectrum were not successful due to an insufficient amount of material.

TLC Characterization of Metabolites. Metabolites A and D2 were confirmed as the parent (maduramicin α) and maduramicin β (mono-O-demethylated at R_{47} of the A-ring), respectively, by 2-D TLC. The first dimension was developed with hexane/acetone (1:1), and the second dimension was developed with MeOH/toluene/EtOAc (3:7:10). The R_f values obtained from both image scanning and vanillin spray were identical for each metabolite and its appropriate reference standard. The minor metabolite B, which was not characterized by MS, gave TLC characteristics very similar to those of maduramicin α and, therefore, was suspected of being an isomer of the parent compound.

Enzyme Hydrolysis of Aqueous Fraction. Incubation of the polar aqueous soluble fraction (7.1% of total radioactivity) with β-D-glucuronidase released 32.4% of the polar metabolites into the organosoluble aglycon fraction. However, most of the enzyme-released organosoluble aglycon fraction failed to resolve into identifiable metabolites when analyzed by TLC.

CONCLUSIONS

Metabolites of maduramicin α were isolated and purified from turkey excreta and characterized by TSP LC/MS, TSP LC/MS/MS, proton NMR, and TLC. The major metabolic pathways for maduramicin α in the turkey were O-demethylation of one or more methoxy groups from either the A- or G-ring followed by hydroxylation at an undefined position. Conjugation with glucuronic acid was also detected as a minor route. This is the first report of multiple O-demethylation and hydroxylation in the metabolism of maduramicin α.

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Received for review February 28, 1990. Accepted August 20, 1990.

Registry No. Maduramicin α, 79356-08-4; maduramicin α metabolite G, 130496-94-5; maduramicin α metabolite C, 131347-86-9; maduramicin α metabolite E, 131273-45-5; maduramicin α metabolite F2b, 131249-91-7; maduramicin α metabolite H, 131273-47-7; maduramicin α metabolite I-1, 131346-27-5; maduramicin β, 79331-53-6.