Novel Degradation Products from the Treatment of Salinomycin and Narasin with Formic Acid

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Salinomycin and narasin (4-methylsalinomycin) upon treatment with HCO_2H furnish the known furanone fragment **3** and the complementary but rearranged fragments **1** and **2** respectively. The structure of **1** has been established by X-ray analysis. Upon being heated under reflux in PhMe, **1** undergoes the retrograde aldol reaction to furnish α,γ-dimethyl-2-furanbutanal (**4**). The furan moiety of **1** is more resistant to electrophilic substitution than expected, but it can be acylated by highly reactive reagents such as $(CF_3CO)_2O$ and $AcOSO_2Me$. Compounds **1** and **2**, the acetyl and trifluoroacetyl derivatives of the former, and the reduction products thereof have no significant anticoccidial activity.

Salinomycin and narasin (4-methylsalinomycin) are important polyether ionophores used to control coccidiosis in broiler chickens throughout the world. Since the 1970s, articles relating to their chemistry have been published, including one mentioning the base-catalyzed degradation of salinomycin to the furanone fragment **3** (see Scheme I).[1,2] We now report the results of our studies on the formic acid degradation of salinomycin in which not only has **3** been isolated but also the complementary (albeit rearranged) piece **1**. Narasin upon treatment with HCO_2H gives **2**. Scheme I indicates a plausible mechanism for these reactions, but omits many of the protonation-deprotonation steps.

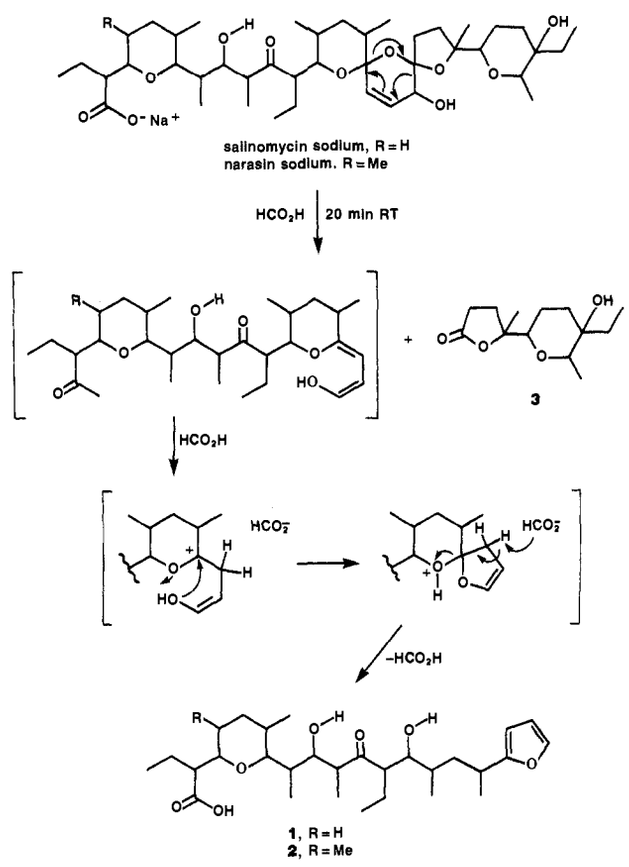
The ^1H NMR spectrum of **1** suggested the presence of a furan ring; this was readily confirmed by its X-ray structure (see supplementary material). While treatment of narasin with HCO_2H did not furnish a crystalline product for X-ray analysis, the ^1H NMR and the ^{13}C NMR spectra of **2** indicated that the structure of **2** is analogous to that of **1**. The important shifts in the ^{13}C NMR spectrum in comparing **2** with **1** correspond to the analogous shifts in the comparison of narasin with salinomycin.[3]

Upon being heated in PhMe under reflux, **1** affords the retrograde aldol reaction product α,γ-dimethyl-2-furanbutanal (**4**, see Scheme II). Further, we have observed that the monosubstituted furan ring of **1** is more resistant to electrophilic substitution than expected (see below).

Neither **1** nor **2** has any significant anticoccidial activity. Therefore, we decided to explore the possibility of modifying these substances to restore this function. With regard to their molecular sizes, **1** and **2** are similar to the smallest of the known natural ionophores, e.g., calcimycin (A-23187) and X-14547A.[4] Further, biologically active ionophores have these structural elements in common: (i) a carboxylic acid group to form a counterion with cations such as sodium and calcium; (ii) several oxygen atoms that can ligate single metal ions; (iii) a cage-like structure in which the hydrophilic elements are located in the interior portion and the lipophilic elements on the exterior; and (iv) a hydrogen-bonding group (usually hydroxy) at a position distal to the carboxy group but able to bond to the carboxy group so as to complete and strengthen the cage-like structure.[4] The X-ray structure of **1** suggests that it fulfills all of these conditions except the last.

Hence, the introduction of a distal hydroxy group in **1** might very well restore anticoccidial activity to this

Scheme I



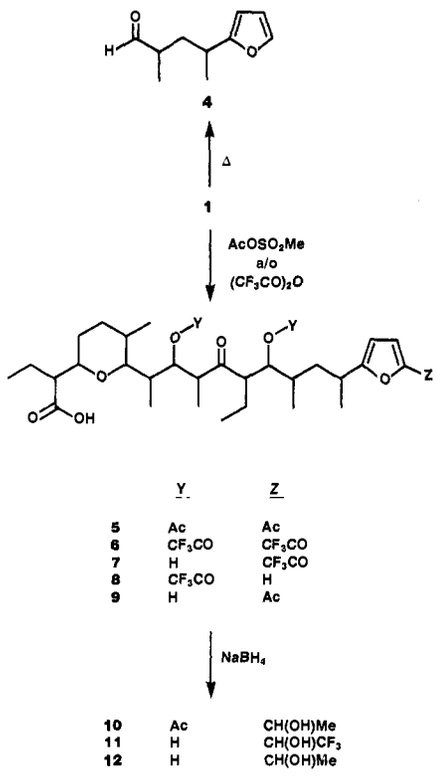
chemical system. We began by attempting to formylate the furan moiety of **1** by using Vilsmeier-Haack conditions. With the introduction of a formyl group, it would then be possible to reduce it with $NaBH_4$ to furnish the corresponding $HOCH_2$ group, thus affording one compound that fulfills the objective. Surprisingly, there was no evidence that the furan system reacted. The only material isolated appeared to be a mixture of formate esters, each with all three furan protons present. In a control experiment, formylation of 2-methylfuran was essentially quantitative and complete within 45 min under comparable conditions.[5] Using conditions more vigorous than this on **1** did not alter the outcome.

The next approach was to investigate acylation of the furan ring of **1**. Similar to the results with the Vilsmeier-Haack reaction, attempts to acetylate **1** on the furan ring with Ac_2O were unsuccessful. More reactive acylating agents, however, gave encouraging results.[6]

(1) Seto, H.; Miyazaki, Y.; Fujita, K.; Otake, N. *Tetrahedron Lett.* **1977**, 2417.
(2) Oikawa, Y.; Horita, K.; Yonemitsu, O. *Tetrahedron Lett.* **1985**, *26*, 1541.
(3) Seto, H.; Yahagi, T.; Miyazaki, Y.; Otake, N. *J. Antibiot.* **1977**, *30*, 530.
(4) Duelser, E. N.; Paul, I. C. In *Polyether Antibiotics*; Westley, J. W., Ed.; Marcel Dekker: New York, 1983; Vol. 2, p 87.
(5) Sumitomo Chemical Co., Ltd. Jpn. Kokai Tokkyo Koho JP 82 56,475; *Chem. Abstr.* **1982**, *97*, 162801s.
(6) Pennanen, S. I. *Heterocycles* **1976**, *4*, 1021.

0022-2623/88/1831-0274$01.50/0

Scheme II



The reaction of **1** with $AcOSO_2Me$[7,8] for 1 h at 0–5 °C gave the triacetyl product **5** (see Scheme II). With an excess of $(CF_3CO)_2O$ at room temperature for 3 h, **1** gave **6**. Treatment of **6** with NH_4OH furnished the deesterified ketone **7**. Further, **1** was treated with 2 molar equiv of $(CF_3CO)_2O$ at −10 to −5 °C for 1.5 h to furnish the bis-[(trifluoroacetyl)oxy] intermediate **8**. Upon subsequent treatment of **8** with $AcOSO_2Me$ and workup there was obtained the monoacetyl derivative **9**. In each case the acylation was confirmed by the presence of a strong, new UV chromophore in the 280–310-nm region.

Ketones **5**, **6**, and **9** were reduced with $NaBH_4$ at room temperature to furnish the corresponding alcohols as mixtures of epimers, i.e., **10**, **11**, and **12**, respectively. The reduction was judged complete by virtue of the disappearance of the strong UV absorption band in the 280–310-nm region. In these processes there was no attempt to purify the reaction mixtures beyond one chromatography procedure. While an excess of $NaBH_4$ was used, this was not sufficient to reduce the carbonyl function on the chain connecting the tetrahydropyran and furan rings. To reduce the latter, it is necessary to heat the reaction solution at 65 °C for 5 h. The crude epimeric alcohols were evaluated as such for anticoccidial activity in chickens.

None of these acylated products nor the mixtures of epimeric alcohols derived therefrom had activity at practical feed levels (120 ppm). At this point we concluded that no simple and easily achievable modification of **1** would confer useful anticoccidial activity to it. Further efforts along this line ceased.

Experimental Section

General Procedures. Unless otherwise noted, when reactions were judged to be complete by TLC analysis, the products were isolated as follows. The reaction solution was poured with vigorous stirring into water. After being adjusted to a pH near neutral, the aqueous mixture was extracted three times with portions of $CHCl_3$, CH_2Cl_2, Et_2O, or AcOEt. The combined extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was then chromatographed. Column chromatographies were performed by using the "flash" technique;[9] silica gel was the adsorbent in each case. ^1H NMR, ^{13}C NMR, and DEPT spectra[10] were obtained in $CHCl_3$-*d* solution. Melting points (mp) are uncorrected.

α-Ethyl-6-[5-ethyl-9-(2-furanyl)-2,6-dihydroxy-1,3,7-trimethyl-4-oxodecyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (1). A solution of 11.5 g (0.015 mol) of salinomycin sodium, 150 mL of 96% HCO_2H, and 75 mL of THF was stirred at room temperature for 30 min. After workup, the earlier chromatography fractions (eluent, AcOEt) furnished 6 g of tacky material containing mainly **1**, while those fractions immediately following contained mainly 5-[5-ethyltetrahydro-5-hydroxy-6-methylpyran-2-yl]dihydro-5-methyl-2*H*-furan-2-one (**3**). The ^{13}C NMR spectrum of **3** was identical with that described in the literature.[2] The combined fractions containing mostly **1** were dissolved in Et_2O. On standing overnight, the solution furnished a mixture of crystals and oil. The mixture was treated with a solution of Et_2O and hexane to dissolve the oil, and the new mixture was filtered. The crystals were washed with four portions of hexane and allowed to dry. There was obtained 2.60 g (34%) of **1**: mp 138–139 °C; $[\alpha]^{23}_D$ −80° (MeOH); UV λ_{max} (MeCN) 230 (ε 2380), 281 nm (440); the x-ray determination of the structure of **1** was made directly on the crystals so obtained; the ^1H NMR spectrum was consistent with the assigned structure; peaks of particular interest are δ 3.61 (d, 1 H), 3.78 (d, 1 H), 4.04 (q, 1 H), 4.12 (d, 1 H), 6.03 (d, 1 H), 6.25 (dd, 1 H), 7.28 (dd, 1 H); ^{13}C NMR δ 7.23 (CH_3), 11.08 (CH_3), 11.96 (CH_3), 12.87 (CH_3), 13.04 (CH_3), 16.11 (CH_3), 16.15 (CH_2), 19.88 (CH_3), 21.30 (CH_3), 22.55 (CH_2), 26.24 (CH_2), 28.14 (CH), 30.97 (CH), 33.70 (CH), 36.46 (CH), 39.69 (CH_2), 48.34 (CH), 48.56 (CH), 57.08 (CH), 70.48 (CH), 71.63 (CH), 74.17 (CH), 74.91 (CH), 103.76 (CH), 109.89 (CH), 140.42 (CH), 160.51 (C), 178.40 (CO), 218.39 (CO); mass spectrum, *m/e* 508 (molecular ion), 382 (less 126), 353 (less 155), 343 (less 165), 325 (less 183), 324 (less 184), 307 (less 201), 108 (parent peak, less 400) among others.

Anal. Calcd for $C_{29}H_{48}O_7$ (508.67): C, 68.47; H, 9.51. Found: C, 68.48; H, 9.51.

α-Ethyl-6-[5-ethyl-9-(2-furanyl)-2,6-dihydroxy-1,3,7-trimethyl-4-oxodecyl]tetrahydro-3,5-dimethyl-2*H*-pyran-2-acetic Acid (2). Narasin sodium (0.5 g, 0.635 mmol) was treated in a manner similar to that described above to furnish **2** as an amorphous solid: yield 158 mg (48%); $[\alpha]^{23}_D$ −87° (MeOH); UV λ_{max} (MeCN) 216 (ε 4200), 284 nm (110); the ^1H NMR spectrum was consistent with the assigned structure; peaks of particular interest are δ 3.55 (d, 1 H), 3.79 (d, 1 H), 3.92 (q, 1 H), 4.16 (d, 1 H), 6.04 (d, 1 H), 6.25 (dd, 1 H), 7.28 (dd, 1 H); the ^{13}C NMR spectrum was consistent with the assigned structure; peaks of particular interest are δ 18.11 (CH_3), 28.89 (CH); mass spectrum, *m/e* 522 (molecular ion), 396 (less 126), 367 (less 155), 357 (less 165), 339 (less 183), 321 (less 201), 108 (parent peak, less 414) among others.

The Na salts of **1** and **2** are not very soluble in water, but their *N*-methylglucamine salts are.

α,γ-Dimethyl-2-furanbutanal (4). A solution of 5.0 g (0.01 mole) of **1** and 200 mL of PhMe was heated under reflux for 2 days. After cooling to room temperature, the solution was evaporated under reduced pressure to furnish an oil, which was then chromatographed (9:1 hexane–AcOEt). There was obtained **4** as an oil in the early fractions: yield 830 mg (51%); $[\alpha]^{23}_D$ −42° (MeOH); ^1H NMR δ 1.10 (d, 3 H), 1.22 (d, 3 H), 1.42 (m, 1 H), 2.18 (m, 2 H), 2.90 (m, 1 H), 5.99 (m, 1 H), 6.23 (m, 1 H), 7.24 (m, 1 H), 9.49 (d, 1 H); ^{13}C NMR δ 13.53 (CH_3), 19.98 (CH_3), 31.04 (CH), 36.83 (CH_2), 44.50 (CH), 104.31 (CH), 110.00 (CH), 141.01 (CH), 158.93 (C), 204.50 (CO); mass spectrum, *m/e* 166 (molecular ion), 108 (less 58, parent peak) among others. Semicarbazone: mp 119–121 °C (from H_2O).

Anal. Calcd for $C_{11}H_{17}N_3O_2 \cdot 0.5H_2O$ (232.28): C, 56.88; H, 7.81; N, 18.09. Found: C, 56.83; H, 7.33; N, 18.11.

(7) Effenberger, F.; Epple, G.; Eberhard, J. K.; Buehler, U.; Sohn, E. *Chem. Ber.* 1983, *116*, 1183.
(8) Karger, M. H.; Mazur, Y. *J. Org. Chem.* 1971, *36*, 528.
(9) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* 1978, *43*, 2923.
(10) Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson.* 1982, *46*, 535.

6-[2,6-Diacetoxy-9-(5-acetyl-2-furanyl)-5-ethyl-1,3,7-trimethyl-4-oxodecyl]-α-ethyltetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (5). A magnetically stirred solution of 4.0 g (7.9 mmol) of **1** and 120 mL of CH_2Cl_2 was cooled to 0–5 °C and then treated with 10.0 g (72 mmol) of $AcOSO_2Me$[7,8] for 1 h. After workup, the residue was chromatographed (3:1 hexane–Me_2CO) to afford **5** as an amorphous solid: yield 1.33 g (27%); UV λ_{max} (MeCN) 218 (ε 2350), 280 nm (10 100); ^1H NMR (selected peaks of interest) δ 2.01 (s, 3 H), 2.03 (s, 3 H), 2.40 (s, 3 H), 3.40 (d, 1 H), 3.92 (d, 1 H), 4.94 (q, 1 H), 5.36 (d, 1 H), 6.15 (d, 1 H), 7.19 (d, 1 H); mass spectrum, *m/e* 634 (molecular ion), 574 (less AcOH), 514 (less two AcOH), and others.

α-Ethyl-6-[5-ethyl-1,3,7-trimethyl-4-oxo-2,6-bis(trifluoroacetoxy)-9-[5-(trifluoroacetyl)-2-furanyl]decyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (6). With magnetic stirring and ice-bath cooling, a solution of 1.00 g (2.0 mmol) of **1** and 20 mL of pyridine was treated with 6.2 g (4.2 mL, 30 mmol) of $(CF_3CO)_2O$. The reaction solution was allowed to warm to room temperature. After 3 h the reaction solution was worked up. The residue was chromatographed (AcOEt) to afford **6** as an amorphous solid: yield 1.15 g (73%); UV λ_{max} (MeCN) 229 (ε 2635), 304 nm (8960); ^1H NMR (selected peaks of interest) δ 3.47 (d, 1 H), 4.00 (q, 1 H), 5.08 (dd, 1 H), 5.51 (d, 1 H), 6.32 (d, 1 H), 7.48 (dd, 1 H). This material was used without further purification in the reaction described below.

α-Ethyl-6-[5-ethyl-2,6-dihydroxy-1,3,7-trimethyl-4-oxo-9-[5-(trifluoroacetyl)-2-furanyl]decyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (7). At room temperature and with magnetic stirring, a solution of 350 mg (0.44 mmol) of **6** and 20 mL of MeOH was treated with 1.0 mL of concentrated aqueous NH_4OH over a period of 1 min. Stirring was continued for 1 h; the solution was evaporated under reduced pressure to furnish an aqueous mixture of organic materials. The aqueous mixture was extracted with $CHCl_3$, the combined extracts were filtered, and the filtrate was dried. The filtrate was then evaporated to furnish **7** as an amorphous solid: yield 200 mg (75%); UV λ_{max} (MeCN) 232 (ε 1860), 307 nm (12500); ^1H NMR (selected peaks of interest) δ 3.68 (d, 1 H), 3.79 (d, 1 H), 3.92 (q, 1 H), 4.13 (d, 1 H), 6.43 (d, 1 H), 7.44 (m, 1 H); high-resolution mass spectrum, *m/e* 604.3191 (molecular ion $C_{31}H_{47}F_3O_8$ requires 604.3223).

α-Ethyl-6-[5-ethyl-9-(2-furanyl)-1,3,7-trimethyl-4-oxo-2,6-bis(trifluoroacetoxy)decyl]tetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (8). Under a N_2 atmosphere and with magnetic stirring, a solution of 2.00 g (3.9 mmol) of **1** and 20 mL of pyridine was cooled to −10 to −5 °C; $(CF_3CO)_2O$ (1.78 g, 1.20 mL, 8.5 mmol) was added. Stirring and cooling were continued for 1.5 h, and then the reaction solution was allowed to warm to room temperature (2 h). After workup and chromatography (3:1 hexane–Me_2CO), there was obtained **8** as an amorphous solid: yield 1.05 g (38%); UV λ_{max} (MeCN) 222 nm (ε 13 700); ^1H NMR (selected peaks of interest) δ 3.50 (dd, 1 H), 3.99 (m, 1 H), 5.10 (dd, 1 H), 5.55 (dd, 1 H), 5.96 (d, 1 H), 6.27 (dd, 1 H), 7.28 (d, 1 H). This material was used without further identification or purification in the reaction described below.

6-[9-(5-Acetyl-2-furanyl)-5-ethyl-2,6-dihydroxy-1,3,7-trimethyl-4-oxodecyl]-α-ethyltetrahydro-5-methyl-2*H*-pyran-2-acetic Acid (9). With magnetic stirring, a solution of 3.70 g (5.28 mmol) of **8** and 70 mL of CH_2Cl_2 was cooled to−5 °C. $AcOSO_2Me$ (3.33 g, 26 mmol) in 15 mL of CH_2Cl_2 was added, and the reaction solution was stirred for 3 h at 0 °C. After workup and chromatography (4:1 hexane–EtOAc), there was obtained **9** contaminated with about 5–10% of **5**: yield 1.66 g (57%); UV λ_{max} (MeCN) 218 (e 3170), 281 nm (13 500); ^1H NMR (selected peaks of interest) δ 2.39 (s, 3 H), 3.54 (d, 1 H), 3.75 (d, 1 H), 3.96 (q, 1 H), 4.08 (d, 1 H, under EtOAc peak), 6.23 (d, 1 H), 7.05 (m, 1 H); high-resolution mass spectrum, *m/e* 550.3516 (molecular ion $C_{31}H_{50}O_8$ requires 550.3505).

Method for Reduction of Ketones 6, 7, and 9. Under a N_2 atmosphere with magnetic stirring, a mixture of 30 molar equiv of $NaBH_4$ and 60 parts of MeOH was cooled to −5 °C. Over a 30-min period, a solution of 1 molar equiv of ketone in MeOH was added dropwise to the mixture. Cooling and stirring were continued for 1 h; the mixture was allowed to warm to room temperature (3.5 h). After workup and chromatography (19:1 $CHCl_3$–MeOH), there was obtained a mixture of the corresponding epimeric alcohols as an amorphous solid. The reaction was judged to be complete when there was no significant absorption in the 280–310-nm region of the UV spectrum.

Biological Evaluation. The compounds described in this article were evaluated for anticoccidial activity by the method of Lynch.[11] The maximum dose employed was 120 ppm of compound in feed, i.e., twice the recommended dose for salinomycin in commercial applications.[12]

Acknowledgment. For evaluating the compounds mentioned in this article for anticoccidial activity we thank our co-workers from the Pfizer Central Research Division: Edward J. Feeney, Dr. Thomas T. Migaki, Deborah Newcomb (nee van Wormer), Dr. Julie A. Olson, and Dr. Anthony P. Ricketts.

Supplementary Material Available: The X-ray structure, tables of the atomic positional and thermal parameters, bond distances, and bond angles for **1** (9 pages). Ordering information is given on any current masthead page.

(11) Lynch, J. E. *Am. J. Vet. Res.* **1961**, *22*, 324.
(12) *Feed Additive Compendium*; Leidahl, R., Ed.; Miller: Minneapolis, MN, 1985; p 304(a).

Additions and Corrections

Bruce E. Maryanoff,* David F. McComsey, Joseph F. Gardocki, Richard P. Shank, Michael J. Costanzo, Samuel O. Nortey, Craig R. Schneider, and Paulette E. Setler: Pyrroloisoquinoline Antidepressants. 2. In-Depth Exploration of Structure–Activity Relationships.

Page 1440. Table IV, compound **23a** (in numerical sequence) was incorrectly shown as **38a**.

Page 1453. Column 2, line 26: "5-HT" should be "S"; lines 31 and 32: The "5-" (before "S_1" and "S_2") should be ignored.

Page 1445. Column 1: "**66b**" (used twice) and "**7b**" should read "**66a**" and "**7a**". Also, "**66b**" on pp 1434 (column 2), 1438 (Table II and footnotes *r* annd *u* to Table II), 1441 (Table IV), and 1443 (column 1) should read "**66a**".