Ivermectin, a New Broad-Spectrum Antiparasitic Agent

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22,23-Dihydroavermectin B_1, ivermectin, derived from avermectin B_1 by selective hydrogenation using Wilkinson's homogeneous catalyst [$(Ph_3P)_3RhCl$], was shown to be a highly effective drug for the treatment of a wide variety of metazoan parasitic diseases in animals.

The discovery of a new family of natural products, the avermectins, was recently reported from these laboratories.[1-4] The avermectins are disaccharide derivatives of pentacyclic, 16-membered lactones, active against helminths and arthropods in doses as low as 10 μg/kg, yet devoid of antibacterial or antifungal activities. Despite their macrocyclic lactone structure, they neither inhibit protein synthesis nor act as ionophores[5] but appear to interfere with the neurotransmission of many invertebrates.[6]

The structures of the eight major naturally occurring avermectins are shown in Figure 1. The a and b series are *sec*-butyl and isopropyl homologues, respectively, whose virtually identical antiparasitic activities eliminated the need for difficult separations for their biological evaluation. All compounds described herein are of the a series, containing up to 20% of the b component.

Examination of Figure 1 reveals that compounds of the **1** series possess an olefinic linkage between carbons 22 and 23, while in the **2** series this linkage is hydrated with the hydroxyl group found at the 23 position. This difference has a profound effect on the conformation of the ring bearing these functionalities and causes subtle changes in bioactivity. For example, it was noted early in our investigations that while avermectin B_1 (**3**) is more active than avermectin B_2 (**4**) upon oral administration, the converse is true when each is administered parenterally. It therefore became an important objective to prepare compounds with the conformation of the **2** series but lacking the 23-hydroxyl substituent.

Chemistry. The desired compounds are the 22,23-dihydro analogues of the avermectin **1** series, and their preparation required the selective reduction of one of their five olefins. Only the target olefin is cis substituted, however, which suggested the use of Wilkinson's homogeneous hydrogenation catalyst $(Ph_3P)_3RhCl$ (**12**), known to be highly sensitive to the steric environment of an olefin. Hydrogenation of **3** for 20 h using **12** in benzene or toluene at 25 °C under 1 atmosphere of hydrogen provided 22,23-dihydroavermectin B_1 (**6**) in 85% yield, together with 3% of 3,4,22,23-tetrahydroavermectin B_1 (**11**). Under the same conditions, avermectin A_1 (**1**) provided 22,23-dihydroavermectin A_1 (**5**) in 92% yield.

The effect of sequential removal of the L-oleandrosyl moieties on anthelmintic activity was also studied. Monosaccharides **7**,[3] **8**,[3] and **9** were prepared in 65–90% yields by acidic alcoholysis in 99:1 2-propanol–concentrated sulfuric acid at 25 °C for 16–18 h. 22,23-Dihydroavermectin B_1 aglycon (**10**) was obtained in 85% yield by stirring **6** in 99:1 methanol–concentrated sulfuric acid at 25 °C for 18 h.

Biological Results and Discussion. All compounds were tested orally against six species of adult gastrointestinal helminths in experimentally infected sheep,[4] following demonstration of their anthelmintic activity in an in vivo laboratory assay.[7] The results are shown in Table I. All had broad spectrum activity with, however, considerable variation in potency, depending on the parasite and compound. In general, compounds of the B series (those containing a 5-hydroxy group) were more potent than those of the A series (with a 5-methoxy group). Differences in potency between the **1** series and the **2** series were more subtle, involving sensitivities of particular parasites. Saturation of the 22,23-olefin had only a small effect on activity, while reduction of both the 3,4- and 22,23-olefins to form **11** considerably lowered activity. Monosaccharides **7**, **8**, and **9** were two- to fourfold less active than the corresponding disaccharides, while 22,23-dihydroavermectin B_1 aglycon (**10**) retained less than one-thirtieth the activity of **6**.

From the preceding data, the most effective compounds were clearly avermectin B_1 (**3**) and 22,23-dihydroavermectin B_1 (**6**). After further study, 22,23-dihydroavermectin B_1 was selected as the more promising candidate on the basis of its overall efficacy by oral and parenteral routes in sheep[8] and cattle,[9] and for its better safety profile.

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Table I. Activity of Avermectin Derivatives against Adult Gastrointestinal Helminths of Experimentally Infected Sheep on Oral Administration

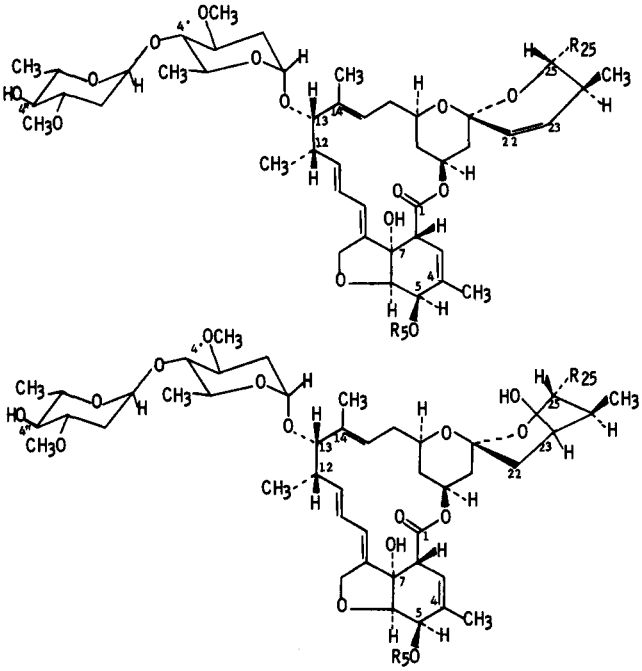
compd	structure[b]	dose, mg/kg	efficacy[a]					
			H.c.[c]	*O.c.*	*T.a.*	*T.c.*[c]	*C.spp.*	*O.c.*
1	A_1	0.1	2	2	0	0	2	0
2	A_2	0.1	3	3	3	3	0	3
3	B_1	0.05	3	3	3	3	3	3
4	B_2	0.1	0	3	3	3	3	3
5	H_2A_1	0.3	3	2	0	1	0	3
6	H_2B_1	0.1	3	3	3	3	3	3
7	B_1MS	0.15	2	2	3	3	3	0
8	B_2MS	0.2	1	1	3	3	3	3
9	H_2B_1MS	0.3	3	3	3	3	2	3
10	H_2B_1AG	3.0	1	2	3	3	1	3
11	H_4B_1	0.2	0	0	1	0	0	3

[a] 0 = <50%, 1 = 50–74%, 2 = 75–90%, 3 = >90% efficacy. Abbreviations used: *H.c., Harmonchus contortus*; *O.c., Ostertagia circumcinta*; *T.a., Trichostrongylus axei*; *T.c., Trichostrongylus colubriformis*; *C.spp., Cooperia spp*; *O.c., Oesophagostomum columbianum*. [b] MS = monosaccharide, AG = aglycon, H_2 = 22,23-dihydro derivative, H_4 = 3,4,22,23-tetrahydro derivative. [c] Benzimidazole resistant.

Table II. Anthelmintic Activity of 22,23-Dihydroavermectin B_1 in Sheep and Cattle

	dose	% removal of adult worms[a]					
		H.spp.	*T.spp.*	*O.spp.*	*C.spp.*	*Oe.spp.*	*D.v.*
sheep	0.1 mg/kg po	>98	>98	>99	>99	>99	
cattle	0.2 mg/kg po	>99	>99	>99	>99	100	100
cattle	0.2 mg/kg im/sc	>99	>99	>99	>98	100	100

[a] Abbreviations used: *H.spp., Haemonchus spp.; T.spp, Trichostrongylus spp.; O spp., Ostertagia spp.; C.spp. Cooperia spp.; Oe.spp., Oesophagostomum spp.; D.v., Dictyocaulus vivaparus.*



1 A_1 R_5 = CH_3 2 A_2 R_5 = CH_3
3 B_1 R_5 = H 4 B_2 R_5 = H
a series R_{25} = $CH(CH_3)C_2H_5$
b series R_{25} = $CH(CH_3)_2$

Figure 1.

22,23-Dihydroavermectin B_1 has been assigned the nonproprietary name ivermectin. Representative anthelmintic data for **6** in cattle and sheep are shown in Table II. Extensive safety and efficacy studies on **6** are currently under way in cattle, sheep, swine, horses, and dogs.

Experimental Section

The 300-MHz ^1H NMR spectra were recorded using a Varian SC-300 NMR spectrometer in the FT mode in $CDCl_3$ with $(CH_3)_4Si$ as internal standard. The mass spectra were recorded using an LKB Model 9000 mass spectrometer at 70-eV ionizing voltage. Both NMR and mass spectra of all compounds are in agreement with the proposed structures and are tabulated as supplementary material (see paragraph at the end of this paper concerning supplementary material). The IR spectra were recorded on neat films using a Perkin-Elmer 267 grating spectrophotometer calibrated against polystyrene film. The UV spectra were recorded in MeOH solutions using a Cary 118 spectrophotometer. The optical rotations were recorded using a Perkin-Elmer 241 polarimeter in a 1-dm cell. The purity of each compound was checked by analytical high-performance LC using a Waters Associates liquid chromatograph on a μBondapak C_{18} column (3.9 mm i.d. × 30 cm) eluted with 85:15 MeOH–H_2O. Preparative high-performance LC was performed on Waters Associates PrepPAK-500/silica cartridges using a Waters Associates Prep LC/System 500 chromatograph. Analytical and preparative TLC was performed using Analtech silica gel GF chromatography plates, and column chromatography was performed on E. Merck silica gel, 70–230 mesh. All lyophilizations were performed using benzene, and all compounds were dried at 25 °C and 0.1 torr for 24 h. Microanalyses for C and H were performed by the Merck Sharp & Dohme Microanalytical Laboratory, Rahway, N.J. 07065, under the direction of J. Gilbert, and agree to within ±0.4% of calculated values.

22,23-Dihydroavermectin A_1 (5). A solution of **1** (2.01 g, 2.27 mmol) and **12** (0.590 g, 0.638 mmol) in toluene (75 mL) was stirred at 25 °C under H_2 (1.1 atm) for 20 h. Evaporation of the solvent and chromatography of the residue on a column of silica gel (250 g) eluted with 3:1 CH_2Cl_2–EtOAc provided, after evaporation, lyophilization, and drying, 1.85 g (92%) of **5** as a white powder, 97% pure: IR 3600–3200 (OH), 1720 (C=O); UV (1.98 × 10^{-5} M) λ_{max} 238 nm (ε 28200), 244 (30800), 252 sh (19900); $[\alpha]_D$ +50.6 ± 0.5° (*c* 0.755, $CHCl_3$), −0.2 ± 0.2° (*c* 0.800, CH_3OH). Anal. ($C_{49}H_{76}O_{14}$) C, H.

22,23-Dihydroavermectin B_1 (6). A solution of **3** (26.5 g, 30.4 mmol) and **12** (10.0 g, 10.8 mmol) in benzene (1.6 L) was shaken at 25 °C under H_2 (16.2 psig) in a stainless-steel vessel. After 20 h, H_2 uptake was 103% of theory. Evaporation of the solvent left a brown glass, which was triturated with 3:2 CH_2Cl_2–EtOAc (15 mL), filtered, and washed with the same solvent (3 × 10 mL). The combined filtrates were evaporated, and the residue was chromatographed on a column of silica gel (2.5 kg) eluted successively with 3:2 CH_2Cl_2–EtOAc (10 L) and 2:3 CH_2Cl_2–EtOAc (14 L), to provide, after evaporation, lyophilization, and drying,

22.6 g (85%) of **6** as an off-white powder. High-performance LC analysis indicated that the product was 95% pure, containing 5% of a mixture of **3** and **11**. Samples of 3.01 and 2.87 g were separately further purified on a column of Sephadex LH-20 (2.8 L) eluted with 7:1:1 hexane–toluene–MeOH to provide 99% pure **6**: IR 3600–3200 (OH), 1715 (C=O); UV (1.89×10^{-5} M) λ_{max} 238 nm (ϵ 27 100), 245 (30 100), 254 sh (19 300); $[\alpha]_D$ +71.5 ± 0.3° (*c* 0.755, $CHCl_3$), −18.1 ± 0.3° (*c* 0.785, CH_3OH). Anal. ($C_{48}H_{74}O_{14}\cdot 0.5H_2O$) C, H.

3,4,22,23-Tetrahydroavermectin B_1 (11). The aforementioned Sephadex LH-20 chromatography of **6** also provided impure **11**, which was purified using preparative TLC (1000 μm of silica gel developed twice with 9:1 toluene–*i*-PrOH). The product was eluted with EtOAc, lyophilized, and dried to provide 231 mg (4%) of **11** as a white powder, 98% pure by high-performance LC: IR 3600–3200 (OH), 1705 (C=O); UV (3.06×10^{-5} M) λ_{max} 238 nm (ϵ 25 800), 244 (29 300), 252 sh (18 900); $[\alpha]_D$ +87.5 ± 1.1° (*c* 0.570, $CHCl_3$), +39.6 ± 0.4° (*c* 0.790, CH_3OH). Anal. ($C_{48}H_{76}O_{14}$) C, H.

22,23-Dihydroavermectin B_1 Monosaccharide (9). To a stirred solution of 99:1 *i*-PrOH–concentrated H_2SO_4 (65 mL) at 25 °C was added solid **6** (500 mg, 0.571 mmol). After stirring for 18 h, the solution was diluted with CH_2Cl_2 (200 mL), neutralized with saturated aqueous $NaHCO_3$ solution, and diluted with enough H_2O to dissolve precipitated salts. The organic layer was separated, washed with brine (100 mL), dried over anhydrous $MgSO_4$, and evaporated. The product was purified using preparative TLC (1000 μm of silica gel developed twice with 19:1 $CHCl_3$–THF). The product was eluted with EtOAc, evaporated, lyophilized, and dried to provide 355 mg (85%) of **7** as a white powder. An analytical sample was prepared by chromatographing 131 mg of **7** on Sephadex LH-20 (150 mL) eluted with 7:1:1 hexane–toluene–MeOH. Evaporation, lyophilization, and drying provided 82 mg of white powder, >99% pure by high-performance LC: IR 3600–3200 (OH), 1720 (C=O); UV (3.29×10^{-5} M) λ_{max} 238 nm (ϵ 26 800), 245 (29 900), 253 sh (19 100); $[\alpha]_D$ +46.6 ± 0.6° (*c* 0.680, $CHCl_3$), −4.3 ± 0.2° (*c* 0.775, CH_3OH). Anal. ($C_{41}H_{62}O_{11}\cdot 0.5H_2O$) C, H.

22,23-Dihydroavermectin B_1 Aglycon (10). To a stirred solution of 99:1 MeOH–concentrated H_2SO_4 (20 mL) at 25 °C was added 2.03 g (2.32 mmol) of **6**. After the solution was stirred for 18 h, the reaction was neutralized by the addition saturated aqueous $NaHCO_3$ solution (20 mL), diluted with Et_2O (20 mL), and enough water was added to dissolve precipitated salts. The layers were separated and the aqueous phase was washed with Et_2O (3 × 10 mL). The combined organic phases were washed with H_2O (25 mL) and brine (25 mL), dried over anhydrous $MgSO_4$, and evaporated. Crude **10** was chromatographed on a column of silica gel (200 g), eluted with 3:1 CH_2Cl_2–EtOAc. Evaporation, lyophilization, and drying afforded 1.03 g (76%) of **10** as a white powder. An analytical sample was prepared using preparative TLC (1000 μm of silica gel developed with 90:10:1 CH_2Cl_2–EtOAc–EtOH). Elution with EtOAc, evaporation, lyophilization, and drying provided pure **10**: IR 3600–3200 (OH), 1712 (C=O); UV (4.03×10^{-5} M) λ_{max} 238 nm (ϵ 25 600), 244 (27 800), 252 sh (18 300); $[\alpha]_D$ +112.1 ± 1.0° (*c* 0.800, $CHCl_3$), +39.7 ± 0.4° (*c* 0.795, CH_3OH). Anal. ($C_{34}H_{50}O_8$) C, H.

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Supplementary Material Available: 300-MHz ^1H NMR and mass spectral data (2 pages). Ordering information is given on any current masthead page.

Synthesis of Potential Inhibitors of Hypoxanthine-Guanine Phosphoribosyltransferase for Testing as Antiprotozoal Agents. 2. 1-Substituted Hypoxanthines

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Evidence indicating that effective in vivo inhibition of hypoxanthine guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8) should produce antiprotozoal activity without significant toxic effects on mammalian hosts prompted syntheses of 1-substituted hypoxanthines bearing functionalized side chains whose groupings might interact with appropriate groupings of HGPRT to form covalent bonds or strong hydrophobic bonds. 3-(Fluorosulfonyl)benzoyl, 4-(fluorosulfonyl)benzoyl, 4-chlorobenzoyl, and bromoacetyl derivatives of two parent amines, 1-(2-aminoethyl)-hypoxanthine and 1-(4-aminobenzyl)hypoxanthine, were synthesized for evaluation in this connection. None of these compounds extended the life span of *Plasmodium berghei* infected mice or showed significant in vitro inhibition of HGPRT from H.Ep.-2 cells, but 1-[2-(bromoacetamido)ethyl]hypoxanthine displayed in vivo activity against *Trypanosoma rhodesiense*.

In the first paper in this series,[1] we reviewed the sizable body of biochemical evidence indicating that the intracellular phosphoribosylation of hypoxanthine, a conversion promoted by hypoxanthine-guanine phosphoribosyltransferase (HGPRT, EC 2.4.2.8), is a vital event in the purine metabolism of protozoal parasites. The parasites are apparently devoid of an alternative biosynthetic route to purine nucleotides, but the cells of their mammalian hosts possess the enzymes of the de novo pathway to purine nucleotides and use the purine phosphoribosyltransferases in secondary ways. The evidence indicates that effective in vivo inhibition of HGPRT should prevent proliferation of protozoal parasites without significant toxic effects on mammalian hosts.

In recent developments in this connection, Kidder and Nolan observed severe inhibition of growth when culture media of *Crithidia fasciulata* and four species of *Leishmania* containing adenine as the purine source were treated with deoxycoformycin, a potent inhibitor of adenine aminohydrolase (EC 3.5.4.2). Deoxycoformycin did not affect growth when hypoxanthine was the purine source.[2] The conclusion that hypoxanthine is the obligatory base for nucleotide synthesis in the organisms studied is the same as that reached by Van Dyke following studies of the relative amounts of incorporation of hypoxanthine, adenine, and adenosine in the nucleic acids of *Plasmodium berghei*.[3] In another recent development, Wang and co-workers found evidence suggesting that the anticoccidial action of 9-(2-chloro-6-fluorobenzyl)adenine is due to inhibition of hypoxanthine transport, possibly

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