reduction of ditelluride **21** (75 mg, 0.3 mmol) to a colorless solution of the tellurol **18** at room temperature, the [11-^{125}I]-1,11-diiodo-1-undecene ([^{125}I]**19**; 2.69 mCi) was added in EtOH, and the mixture was stirred for 1 h. The product was worked up in the usual manner as for [^{125}I]**15** and chromatographed on SiO_2 to give 547 μCi of [^{125}I]**23**, homogeneous upon thin-layer radiochromatographic analysis, R_f 0.50 (C_6H_6). Basic hydrolysis in the usual manner gave 532 μCi (97%) of 18-[^{125}I]iodo-7-tellura-17-octadecenoic acid ([^{125}I]**24**) showing a single radioactive component on TLC, R_f 0.27 (4% MeOH–$CHCl_3$). The product was stored under argon in sealed amber break-seal tubes at 0 °C until further use.

13-[^{123m}Te]Telluraheptadecanoic Acid (13-[^{123m}Te]THDA). The ^{123m}Te-labeled fatty acid was prepared by basic hydrolysis of the purified fatty acid methyl ester obtained by simultaneous butyl bromide and methyl 12-bromododecanoate alkylation of $Na_2{}^{123m}Te$ generated by $NaBH_4$ reduction of ^{123m}Te. The details of the chemical synthesis and properties of this tellurium fatty acid will be reported elsewhere.

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A Polymeric Drug for Treatment of Inflammatory Bowel Disease[1]

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Sulfasalazine (SASP) consists of salicylic acid azo linked at the 5-position to a pyridine-containing sulfonamide. This drug, currently used in inflammatory bowel disease treatment, is reductively cleaved by anaerobic bacteria in the lower bowel to 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP). Recent reports indicate that 5-ASA is the active therapeutic moiety and that SP is responsible for a variety of adverse clinical side effects. Water-soluble polymer **7**, which contains salicylate residues azo linked at the 5-position to an inert polymer backbone, has been synthesized for the site-specific reductive release of 5-ASA in the lower bowel. Preparations of **7** deliver (chemical reduction) >1.96 mmol of 5-ASA/g of polymer. In vitro studies with the polymer in anaerobic rat cecal bacteria demonstrated a reduction rate of approximately 1 μequiv of azo bond h^{-1} (mL of cecal content)$^{-1}$. A pharmacokinetic comparison of polymer and SASP showed similar deliveries of 5-ASA and metabolites to the lower bowel, blood, and urine of orally dosed rats. Polymer **7** proved more active than SASP or 5-ASA in the guinea pig ulcerative colitis model. Potential therapeutic advantages of **7** include nonabsorption/nonmetabolism in the small intestine, direct 5-ASA release at the disease site, and nonabsorption/nonmetabolism of the reduction-released carrier polymer.

Sulfasalazine (salicylazosulfapyridine, Azulfidine, SASP)[3] is the most effective of various sulfonamides for the treatment of ulcerative colitis and has been used clinically for over 30 years.[4] Results of recent studies suggest that SASP may also be effective for treatment of Crohn's disease of the colon.[5] SASP (**1**) consists of salicylic acid linked to a pyridine-containing sulfonamide by an azo bond at the 5-position. The disposition and metabolism (Scheme I) of the drug in man have been well studied.[6-8] When administered orally, about 30% of the intact drug is absorbed from the small intestine. The absorbed portion undergoes enterohepatic circulation with 2–10% excreted intact in the urine, and the remainder returned to the gut via the bile. SASP in the gut travels to the lower bowel where anaerobic bacteria reductively cleave the azo bond, producing sulfapyridine (SP, **2**) and 5-aminosalicylic acid (5-ASA, **3**).[7] Approximately one-third of the 5-ASA is absorbed and excreted in urine as *N*-acetyl derivative **6**, with the remainder being excreted unchanged in the feces. SP is absorbed and undergoes N-acetylation (to **4**) and 5′-hydroxylation (to **5**). These two compounds are further metabolized and excreted in the urine as glucuronide conjugates.[9,10]

Limitations to the use of SASP are the development of adverse gastrointestinal, hematological, and generalized side effects, or more serious reactions, including agranulocytosis, toxic epidermal necrolysis, paresthesia, hepatotoxicity, pancreatitis, pulmonary disease, and male infer-

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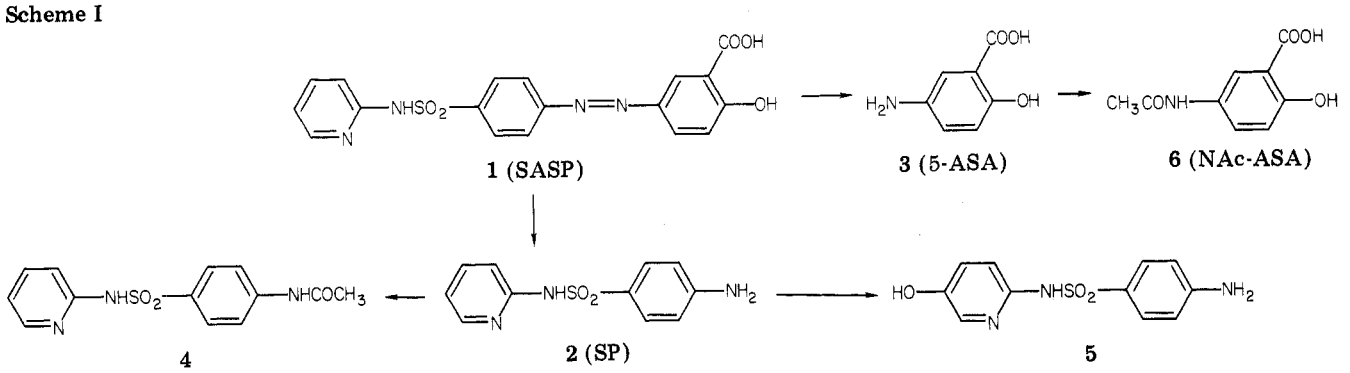
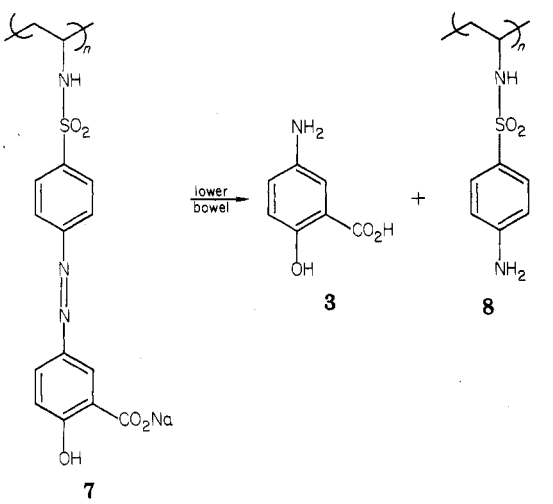
Scheme I



tility.[4,7,11,12] The toxic symptoms ascribed to SASP have been correlated with high serum concentrations of SP (>50 μg/mL) and with decreased ability to acetylate SP.[10,13] The toxic symptoms have not been correlated with SASP or 5-ASA serum concentrations.

The therapeutic action of SASP could, in theory, be related to the intact drug, to either the antibacterial (SP) or antiinflammatory (5-ASA) cleavage product, or to some combination of these materials. Azad Khan et al. administered enemas of SASP, SP, and 5-ASA to patients with ulcerative colitis.[14] About 75% of those receiving SASP or 5-ASA improved, compared to only 38% of those receiving SP. Van Hees et al. showed complete remission of idiopathic proctitis in 60, 13, and 27% of patients given, respectively, suppositories of 5-ASA, SP, or placebo.[15] Klotz et al. confirmed the activity of 5-ASA suppositories in patients with ulcerative colitis and Crohn's disease.[16] Sharon et al. obtained results indicating prostaglandin E_2 (PGE_2) possibly mediates the inflammatory response in ulcerative colitis and showed 5-ASA to be twice as effective as SASP or SP for the inhibition of PGE_2 synthesis.[17] These studies strongly suggest 5-ASA (**3**) to be the therapeutic moiety of SASP.[18]

If this hypothesis is correct, the toxic sulfapyridine portion of SASP serves primarily to limit intestinal absorption until 5-ASA is generated by colonic bacterial reduction. Water-soluble polymer **7**, which contains sodium salicylate residues linked at the 5-position by an azo bond to an inert polysulfanilamide backbone, has been synthesized for the site-specific metabolic release of 5-ASA in the lower bowel and is presently under investigation as a potential chemotherapeutic agent for the treatment of inflammatory bowel disease.[19] The advantages of polymer **7** include nonabsorption from the small intestine, elimination of SP-produced side effects, and nonabsorption of polymeric cleavage product 8.[20] We report here the synthesis and chemical characterization of salicylate polymer **7**. We also report the results of biological studies designed to assay the reductive cleavage of polymer **7** by anaerobic rat cecal bacteria in vitro; quantitate and compare the delivery, by both polymer **7** and SASP, of 5-ASA and its metabolites to the lower bowel, blood, and urine of orally dosed rats; and determine the effect of polymer **7** on carrageenan-induced ulcerative colitis in guinea pigs.



Experimental Section

Chemistry. General. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Ultraviolet and visible spectra were obtained with a Cary Model 118 spectrophotometer. The ^1H NMR spectrum was recorded with a Varian T-60A spectrometer and is reported as parts per million (δ) relative to internal tetramethylsilane. ^{13}C NMR spectra (0–200 ppm, proton decoupled) were recorded with a Varian XL-100 instrument by the NMR Laboratory, Stanford University, Stanford, CA, with internal dioxane (67.4 ppm) for calibration. Combustion analyses were performed by the Microanalytical Laboratory, Stanford University. Molecular weights were determined by gel permeation chromatography and were calculated from peak elution volumes recorded relative to either sulfonated polystyrene standards (M_p^{PPS}), eluting with 0.05 M aqueous phosphate buffer (pH 7.0),[21] or polystyrene standards (M_p^{PS}), eluting with 0.01 M LiBr in dimethylformamide.[22] Si-

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lanized glass columns were employed for both methods.

Polymeric salicylate 7 was purified by aqueous ultrafiltration.[23] A H1P10 hollow fiber cartridge (molecular weight cutoff 1×10^4) was employed (Amicon Corporation, Lexington, MA) in conjunction with a variable-speed magnetically coupled centrifugal pump (Micropump, Model 101-411-088, Cole-Parmer Instrument Co., Chicago, IL). Direct functional analyses of the polymers were conducted in Dynapol's analytical laboratories as follows: the amine content of poly(vinylamine hydrochloride) (**10**) was determined by titration with tetrabutylammonium hydroxide in dimethyl sulfoxide; residual backbone amine content in Schotten–Baumann product **12** was determined by a modified Van Slyke method in tetrahydrofuran–water (3:1);[24] the aromatic amine content of polymeric sulfanilamide **13** was determined by diazotization, followed either by pyrolysis GC for N_2 or by coupling to *N*-(1-naphthyl)ethylenediamine (Aldrich Chemical Co., Milwaukee, WI) and reductive azo bond titration ($CrCl_2$). Experimental details of the analytical methodology are to be published elsewhere.

Poly(*p*-acetamido-*N*-vinylbenzenesulfonamide) (12). Poly(vinylamine hydrochloride) (**10**) was prepared from poly(*N*-vinylacetamide) of M_p^{PS} 1.2×10^5 as previously reported and isolated by precipitation into isopropyl alcohol.[25] A 2-L, four-neck flask, equipped with overhead stirrer, 125-mL dropping funnel filled with 8 N NaOH, pH probe, and Ar inlet, was charged with 42.0 g (0.454 equiv amine) of poly(vinylamine hydrochloride), 420 mL of H_2O, 50 mL of 8 N NaOH, and 210 mL of tetrahydrofuran (THF). After stirring for several minutes, a homogeneous solution of pH 10 was obtained. With vigorous stirring, 44.2 g (0.189 mol) of powdered sulfonyl chloride **11** (Aldrich Chemical Co.) was added, and the pH was maintained at 9–10 by base addition as necessary for 5 min. A second portion of sulfonyl chloride (44.2 g) was then added, followed by 210 mL of THF. After an additional 15 min at pH 9–10, a third equal portion of sulfonyl chloride was added, followed by 210 mL of THF, and the pH was maintained at 10–11 until no further reaction was observed (stable pH, 60 min).

The crude reaction mixture was transferred to a 3-L flask, and the THF was removed by rotary evaporation [35 °C (20 mm)]. Schotten–Baumann product **12** precipitated as an easily filterable, light-tan brittle solid. The yield was 98.0 g (85% based on a product sulfur content of 3.92 mequiv/g) after thorough water washing and drying. A small portion of the crude product was dissolved in 2-methoxyethanol and precipitated into 10 volumes of rapidly stirred H_2O. Filtration afforded **12** as a white powder, which was shown to contain 0.03 mequiv/g of aliphatic amine. A ^{13}C NMR spectrum, obtained in dioxane–water, showed only the sulfonamide residue. Elemental analysis data gave (mequiv/g basis): N/S, 2.04 (calcd 2.00); C/S, 10.50 (calcd 10.00); C/N 5.13 (calcd 5.00).

Poly(*p*-amino-*N*-vinylbenzenesulfonamide hydrochloride) (13). To a 2-L, three-neck flask, equipped with mechanical stirrer and reflux condenser, was added 53.6 g (0.210 equiv based on sulfur content) of crude **12** (prepared above), 800 mL of H_2O, and 111 mL (1.33 mol) of 12 N HCl. The mixture was stirred at reflux for 6 h, and the clear solution was cooled. A 10-mL aliquot was withdrawn for purification and analysis, while the remainder was used for diazotization.

The reaction aliquot was precipitated by addition to isopropyl alcohol, and the resultant solid was isolated and dried. Aromatic amine determination for the purified product indicated the hydrolysis to be 99% complete. A ^{13}C NMR spectrum, obtained in dioxane–aqueous HCl, showed only the desired polysulfanilamide residue. Elemental analysis data gave (mequiv/g basis): N/S, 2.06 (calcd 2.00); C/S, 8.32 (calcd 8.00); C/N, 4.03 (calcd 4.00).

Polymeric Salicylate 7. A 3-L, three-neck flask, fitted with an overhead stirrer, was charged with the solution of polysulfanilamide hydrochloride **13** (0.210 equiv) as obtained directly from the preceding step and 1500 mL of H_2O. The flask was fitted with a funnel extending below the surface of the solution and with a gas exit tube. With vigorous stirring, a solution of 21.5 g (0.253 mol) of KNO_2 in 7.8 mL of H_2O was added via the funnel as rapidly as possible, and the clear polymeric diazonium salt solution was immersed in an ice bath and stirred for 3 min (positive KI–starch test).

A 10-L battery jar, fitted with overhead stirrer, thermometer, pH probe, and 250-mL dropping funnel filled with 8 N NaOH, was charged with 87.1 g (0.631 mol) of salicylic acid (Aldrich Chemical Co.), 3000 mL of H_2O, and 158 mL (1.26 mol) of 8 N NaOH. After the clear solution (pH 13.35) was stirred for 5 min, ice was added to lower the temperature to 15 °C, and addition of the diazonium salt (via peristaltic pump) was begun. Throughout the addition (20 min), the pH was maintained at 13.1–13.3 by addition of 8 N NaOH (170 mL, 1.36 mol), and the temperature was maintained at 17–18 °C by ice addition (final volume 7.0 L).

The deep-red solution was stirred for 60 min at ambient temperature, filtered through a coarse-frit filter, and ultrafiltered. The ultrafiltration was conducted by first concentrating the reaction mixture to 4 L volume and then ultrafiltering for 8×4-L diavolumes with 0.05% aqueous NaCl. After a final 2×4-L diavolume ultrafiltration with H_2O, the solution was concentrated and freeze-dried to afford 66.9 g of polymeric salicylate **7** as an orange solid. The product exhibited the following: M_p^{PSS} 2.5×10^3; UV λ_{max} (H_2O) 354 nm; a 44.2 $(g/L)^{-1}$ cm^{-1}; 1.87 mequiv of azo bond/g by $CrCl_2$ titration (not corrected for H_2O content). Elemental analysis data gave (mequiv/g basis): N/S, 3.00 (calcd 3.00); C/S, 14.7 (calcd 15.0); C/N, 4.89 (calcd 5.00). A detailed discussion of the structure of **7** may be found under Results.

5-Acetamidosalicylic Acid (6). The *N*-acetyl derivative (*N*-Ac-ASA) was prepared in 50% yield from 5-aminosalicylic acid (Aldrich Chemical Co.) and excess acetic anhydride (25 °C, 36 h), followed by treatment with 1 equiv of NaOH (H_2O, 25 °C, 2 h) to remove the oxygen-bound acetyl, acidification (HCl), extraction (EtOAc), and recrystallization (H_2O). The product exhibited the following: mp 218–220 °C; ^1H NMR (dimethyl sulfoxide-d_6) δ 6.7–8.0 (complex m, 3, aromatic), 2.00 (s, 3, acetyl). Anal. ($C_9H_9NO_4$) C, H, N.

Biology. Chemicals. The following were obtained from the indicated supplier: acetic anhydride, hydrochloric acid, and monobasic potassium phosphate from J. T. Baker Chemical Co. (Phillipsburg, NJ); 4-methyl-2-pentanone from Mallinckrodt Chemical Co. (St. Louis, MO); bacterial type I β-glucuronidase (7.5×10^4 units/g), glucose 6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP), and type XV glucose-6-phosphate dehydrogenase from Sigma Chemical Co. (St. Louis, MO); 5-aminosalicyclic acid (**3**) from Aldrich Chemical Co. (Milwaukee, WI); benzylviologen from ICN (Irvine, CA); sulfasalazine (**1**) from Pharmacia AB (Uppsala, Sweden); and degraded carrageenan from Marine Colloids (Rockville, ME).

Animals. Female Sim:(SD)fBr rats (Simonsen Laboratories, Gilroy, CA) weighing 200–300 g were acclimated to metabolism cages for 72 h before dosing. Rats received Purina Laboratory Chow (Ralston Purina Co., St. Louis, MO) and tap water ad libitum throughout the study. Groups of approximately 20 Hartley-strain male guinea pigs (Elm Hill Breeders, Chelmsford, MA) weighing 242–265 g were housed five per cage and maintained on Purina guinea pig chow (Ralston Purina Co.) and water ad libitum prior to carrageenan administration in the drinking water.

In Vitro Metabolism. The general methodology for the assay of azo bond reduction by rat cecal bacteria and their enzymic extracts in vitro has been described.[26] About 3 g of cecal contents was removed from a freshly sacrificed rat, suspended (1.0 g fresh weight/25 mL) in VPI diluent,[27] and filtered through glass wool

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under N_2 (O_2 free). To 5.0 mL of cell suspension (contained in screw-cap tubes) were added 0.05 mL of 20 wt % α-D-glucose, 0.5 mL of 0.001 M benzylviologen or distilled H_2O, and 2.5 mL of solutions (0.6 μequiv of azo bond/mL) of polymer **7** or the previously investigated[28] polymeric analogue of FD & C Yellow No. 6 (Poly S-119, Aldrich Chemical Co.). The tubes were degassed (O_2-free N_2), sealed tightly with Hungate stoppers, and incubated (35 °C, 48 h). The periodic removal of 1.0-mL samples enabled assay of azo bond reduction by measurement of the absorbance at the λ_{max} of the polymers (354 and 475 nm, respectively, for polymer **7** and the control).

An anaerobic cell-free extract (CFE) was prepared by the sonic disruption (Braunsonic Model 1510, 50–60 W, 10 min, 4 °C) of a suspension of rat cecal bacteria (1.0 g fresh weight/10 mL of VPI diluent), followed by centrifugation (1.3×10^4 g, 20 min). The CFE was decanted into a screw-capped tube and stored at 0 °C. Reduction was spectrophotometrically (Varian Techtron Model 635) monitored with a modified anaerobic cuvette with a side arm that has been previously described.[26] The cuvette was charged with 0.2 mL (0.12 μequiv of azo bond) of polymer solution, 0.5 mL of CFE, 0.02 mL of 0.001 M benzylviologen, 1.0 mL of 0.25 M Krebs–Ringer phosphate buffer (pH 7.4), 0.05 mL (0.75 μmol) of NADP, 0.05 mL (2.5 μmol) of glucose 6-phosphate, and 1.0 mL of H_2O and then placed in the thermostated (37 °C) cell holder of the spectrophotometer. The cuvette was degassed (O_2-free N_2), and reduction was initiated by the addition of 10 μL (2 units) of glucose-6-phosphate dehydrogenase. The initial (linear) reduction rate was calculated in microequivalents of azo bond reduced per hour per milliliter of CFE.[26]

In Vivo Metabolism. Nonfasted rats received a single oral dose of either 20 mg (37 μequiv of azo bond) of polymer **7** or 16 mg (40 μmol) of SASP (**1**) as, respectively, aqueous solutions or suspensions. Blood, urine, and feces were collected from experimental rats and from controls at intervals to 96 h after dosing. The quantitation of 5-ASA and its metabolites in the biological samples was based upon a fluorescence assay for *N*-Ac-ASA (**6**), as this material affords much better spectral linearity in the 0.5–500 nmol/g sample range than does 5-ASA. This procedure, modified from that reported by Hansson,[29] provided overall quantitation of the three salicyclic acid based metabolites (5-ASA, *N*-Ac-ASA, and 5-ASA glucuronide) of polymer **7** and SASP. Control samples of *N*-Ac-ASA were employed to generate standard extraction curves.

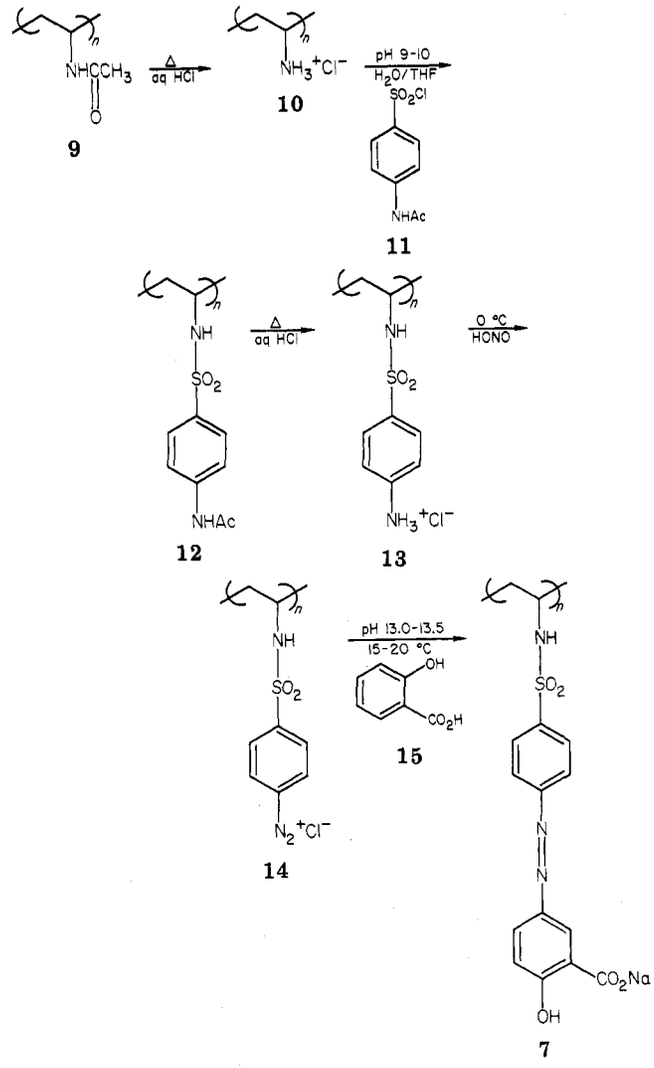
The extraction/quantitation was conducted by adding 1.0 mL of 0.1 M phosphate buffer (pH 6.9) and 110 units of β-glucuronidase to 1.0 g of biological specimen and incubating (37 °C, 18 h). Samples were then treated with acetic anhydride (25 μL), 1 M HCl (1.0 mL) and 4-methyl-2-pentanone (5.0 mL) and extracted on a mechanical shaker (20 min at 240 cycles/min). The organic layer was separated by centrifugation, and 4.0 mL was removed and extracted with phosphate buffer (3.0 mL, pH 6.0). Following a second centrifugation, the organic layer was discarded, and the aqueous portion was assayed in an Aminco-Bowman spectrofluorimeter (Model SPF-125). Uncorrected excitation and fluorescence wavelengths of 295 and 460 nm, respectively, were employed. Identically treated controls were run in parallel with each set of biological samples.

Guinea Pig Studies. Separate groups of 20 animals were treated (gavage) twice daily with solutions or suspensions of 24 mg (60 μmol) of SASP (**1**), 15 mg (60 μmol) of SP (**2**), 30 mg (56 μequiv of azo bond) of polymer **7**, or 10 mg (65 μmol of 5-ASA (**3**) for 3 days. All animals, including a control group that received no treatment, were then given 5 wt % sterile degraded carrageenan solutions as their drinking water (sole fluid source), and the treatment regimen was continued. Animals were observed twice daily for evidence of diarrhea, and general appearance and fluid consumption were noted. Each animal was weighed at the outset of the experimental regimens and at 3-day intervals thereafter.

The study was terminated after 24 days of carrageenan administration because of obvious severe disease in the control group. At necropsy, the cecum and colon of each animal were examined by transmitted light under a dissecting microscope. Animals showing evidence of mucosal ulceration, hemorrhage, or other anatomical abnormalities of the lower bowel were graded positive for gross abnormality, and a detailed description of the abnormality was recorded. No attempt to score disease severity was made at necropsy. Tissue samples obtained from the midcecum of each animal were processed for histological evaluation.

Disease severity in each group was quantitated by using the histopathological criteria of (a) polymorphonuclear cells found in the mucosal epithelium, (b) edema, (c) crypt abscess, (d) crypt loss, (e) epithelial thinning, and (f) frank ulceration.[30,31] Each criterion present was given a score of 1 point. Total points for each group were averaged, and a Student's *t* test of the mean vs. the control group was performed. Results of the histological evaluation were reviewed "blind" by one of us (A.B.O.) and by a board-certified veterinary pathologist.

Scheme II



Results

Chemical Synthesis. The preparation of polymeric sodium salicylate **7** is outlined in Scheme II. . The synthetic sequence to polymeric diazonium salt **14** was developed earlier for the preparation of sulfonate-solubilized polymeric azo dyes and has been reported in detail.[25,32]

(28) T. Honohan, F. E. Enderlin, B. A. Ryerson, and T. M. Parkinson, *Xenobiotica*, **7**, 765 (1977).

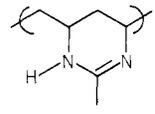
(29) K. A. Hansson, *Acta Pharm. Suec.*, **10**, 153 (1973).

(30) A. B. Onderdonk, J. A. Hermos, J. L. Dzink, and J. G. Bartlett, *Gastroenterology*, **74**, 521 (1978).

(31) (a) H. G. Boxenham and W. Dairman, *Drug Dev. Ind. Pharm.*, **3**, 121 (1977); (b) A. B. Onderdonk and J. G. Bartlett, *Am. J. Clin. Nutr.*, **32**, 258 (1979); (c) J. Watt, S. N. Marcus, and A. J. Marcus, *J. Pharm. Pharmacol.*, **32**, 873 (1980).

The coupling of **14** to salicylic acid (**15**) was found to proceed best if conducted at 15–20 °C and pH 13.0–13.5 with 2–3 equiv of salicylic acid. These reaction conditions (previously reported couplings were conducted at 0–5 °C with 1.1 equiv of coupling agent) were necessary to counteract the sluggish coupling kinetics of salicylic acid. Polymer **7** is easily purified by aqueous ultrafiltration[23] and can be isolated as an orange solid by lyophilization. The polymer exhibits solubility properties similar to analogous sulfonate-solubilized polymers, although precipitation occurs on carboxyl-group protonation (pH < 4.5).

Polymer Composition. Determination of the composition of **7** involves both confirmation/quantitation of the desired mer group and identification/quantitation of spurious mer groups resulting from either incomplete or side reactions during the synthetic sequence.[33] Initial focus must therefore be on the composition of each of the key polymeric intermediates (**10** and **12–14**). Poly(vinylamine hydrochloride) (**10**), as generated by the hydrochloric acid hydrolysis of poly(*N*-vinylacetamide) (**9**), is not an absolute homopolymer. The product contains amidine groups (represented by **16**) accounting (based on ^{13}C

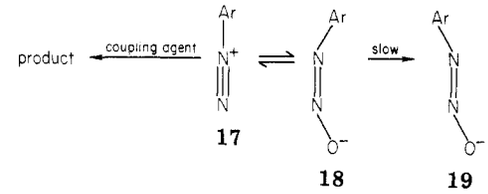


16

NMR, titration, and elemental analysis data) for about 4 out of every 100 mer units.[34] These groups result from acid-catalyzed condensation of adjacent amine and acetamido groups[35] and may be avoided by use of alkaline hydrolysis media.[36] *N*-Vinylacetamide units can also be present in **10** if the hydrolysis of **9** is not carried to completion.

Assay for residual backbone vinylamine residues in Schotten–Baumann product **12** is readily accomplished by a modified Van Slyke determination.[24] Essentially complete amine substitution is attained in the condensation with the use of 1.25 equiv of sulfonyl chloride and careful control of pH (9–10) and temperature (15–20 °C). Without careful control of the reaction conditions, **12** will assay for 5 mer % (or more) residual backbone amine groups. ^{13}C NMR spectra of polysulfanilamide **13** show only the desired mer group, plus (any) unreacted backbone groups carried into this step. Aromatic amine in **13** can be determined by either diazotization, followed by pyrolysis GC with thermal conductivity detection for N_2, or by diazotization and coupling to *N*-(1-naphthyl)ethylenediamine,

Scheme III



followed by reductive azo bond titration with $CrCl_2$. The agreement between both analyses is good (the pyrolysis value is typically 3% lower), and the results show that the hydrolysis of **12** is 99% complete under the reaction conditions employed.

Analysis of polymer **7** has primarily involved quantitation of the desired (5-azo-leashed salicylate) mer group. Coupling at the 3-position of salicylic acid (**15**) is not favored but will occur to a small extent, ultimately affording 3-aminosalicylic acid (3-ASA) on cleavage. The desired mer group in **7** can be quantitated in two ways. The first method involves cleavage of all azo bonds with excess sodium hydrosulfite ($Na_2S_2O_4$) and quantitation of the liberated ASA isomers by UV spectroscopy, followed by determining the ASA isomer ratio by reverse-phase high-performance liquid chromatography (C-18 column) with cetyltrimethylammonium bromide for ion pairing. A second method involves reductive azo bond titration of **7** with $CrCl_2$. Reducible diazotate groups present (vide infra), which interfer with this titration, are destroyed by a pretreatment with hot ethanolic HCl. The agreement between the two analyses is excellent, with the titration affording 1% higher results on average. Typical preparations of polymer **7** are capable of delivering (by chemical reduction) >30 wt % 5-ASA (>1.96 mmol/g) and 0.3–0.4 wt % 3-ASA.

Although the remaining groups present in polymer **7** have not been completely characterized or quantitated, there is sufficient evidence to permit reasonable conclusions as to their nature. Analytical results for polymers **10**, **12**, and **13** show that, with the exception of trace residual backbone *N*-vinylacetamide, vinylamine, and amidine residues, polysulfanilamide **13** is essentially a homopolymer. Any major auxiliary groups present in **7** must necessarily be formed in the diazotization and coupling steps. The diazotization appears quantitative, since no residual sulfanilamide units or side products proved detectable.[37] The addition of a diazonium salt solution to an alkaline solution of coupling agent produces the reactions pictured in Scheme III. At high pH the diazonium ion (**17**), which couples to yield product, is in facile equilibrium with *cis*-diazotate **18** (derived from attack of hydroxide on **17**). The *cis*-diazotate slowly isomerizes to more stable *trans*-diazotate **19**, which does not readily revert to **17** at high pH.[38] Any *trans*-diazotate produced during the coupling of **14** will appear in the product. High

(32) (a) D. J. Dawson, R. D. Gless, and R. E. Wingard, Jr., *Chem. Technol.*, **6**, 724 (1976); (b) D. J. Dawson, R. D. Gless, and R. E. Wingard, Jr., *Polym. Prepr., Am. Chem. Soc., Div. Polym. Chem.*, **17**(2), 779 (1976); (c) N. M. Weinshenker, *ibid.*, **18**(1), 531 (1977); (d) N. M. Weinshenker, in "Polymeric Drugs", L. G. Donaruma and O. Vogl, Ed., Academic Press, New York, 1978, pp 17–37; (e) W. J. Leonard, Jr., in "Polymeric Delivery Systems", R. Kostelnik, Ed., Gordon and Breach, New York, 1979, pp 269–292; (f) D. J. Dawson, *Aldrichimica Acta*, **14**(2), 23 (1981).

(33) A mer unit is a monomeric unit of a polymer. The term "mer %" expresses the fraction of repeating backbone units (i.e., two-carbon units) substituted by a given residue and is analogous to "mol %", except that the units are covalently bound together.

(34) G. V. McGarraugh, R. Phillips, and D. J. Dawson, unpublished results.

(35) R. H. DeWolfe, in "The Chemistry of Amidines and Imidates", S. Patai, Ed., Wiley, New York, 1975, pp 350–373.

(36) D. J. Dawson and P. J. Brock, unpublished results.

(37) A possible side reaction during diazotization is reaction of (any) residual backbone vinylamine groups with HNO_2. To evaluate this possibility, we subjected a sample of 80:20 poly(*N*-vinylacetamide-*co*-vinylamine) to conditions identical with those employed for the diazotization of **13**. Analyses (^{13}C NMR and proton titration) showed 96% of the amine remaining. The large excess of HCl (6 equiv) used in this step apparently tightly protonates, and thus protects, (any) residual aliphatic amine. We thank M. Kronstadt for this experiment.

(38) The *trans*-diazotate will revert to diazonium ion in acidic media. An excellent discussion of the equilibria involved in the coupling of diazonium salts may be found in H. Zollinger, "Azo and Diazo Chemistry. Aliphatic and Aromatic Compounds", Interscience, New York, 1961, pp 38–56.

Table I. Recovery of 5-ASA and Metabolites After Oral Administration to Rats[a,b]

test agent	rat no.	% of administered dose: urine	% of administered dose: feces	% of administered dose: total	peak serum concn, nmol/mL
polymer 7	1	6.7	5.3	12.0	1.8
	2	9.5	9.4	18.9	2.6
	3	7.7	22.0	29.7	3.0
	mean ± SEM	8.0 ± 0.81[c]	12.3 ± 5.0	20.2 ± 5.1	2.5 ± 0.35[c]
SASP	4	3.0	7.7	10.7	7.9
	5	3.2	14.0	17.2	4.0
	6	5.0	11.4	16.4	4.8
	mean ± SEM	3.7 ± 0.64	11.0 ± 1.8	14.7 ± 2.0	5.6 ± 1.2

[a] Experiment based on 37 μequiv of azo bond of polymer 7 and 40 μmol of SASP. [b] 5-ASA and metabolites were converted to *N*-Ac-ASA (6) for spectrofluorometric assay. [c] $p < 0.05$.

Table II. Guinea Pig Physical Findings and Necropsy Results[a]

group[b]	wt loss, g	% of incidence: diarrhea	% of incidence: mortality[c]	% of incidence: abnormal at necropsy[d]
control	29	95	15	95
SASP	44	100	40	90
SP	17	80	15	90
5-ASA	12	83	28	83
polymer 7	37	85	25	85

[a] Experiment based on 60 μmol of SASP, 60 μmol of SP, 65 μmol of 5-ASA, and 56 μequiv of azo bond of polymer 7. [b] All groups contained 20 animals, except the 5-ASA group which contained 18. [c] Prior to necropsy. [d] All animals, including those which died prior to the termination of the study, were evaluated.

reductive titration values for acid untreated samples indicate the presence of this group, as does the slow formation of red color when 7 is dissolved in acidic media in the presence of *N*-(1-naphthyl)ethylenediamine.

Another side reaction of diazonium salt 14 is loss of nitrogen to afford an aryl carbonium ion, which will then react with prevalent nucleophiles (such as hydroxide or chloride). Elemental analysis data for most preparations of 7 afford N/S mol ratios lower than 3 (generally 2.90–2.95), showing loss of nitrogen by 14. Low levels of chlorine (10–20 ppm) by elemental analysis and high values obtained in the titration of 7 by base indicate the presence of phenolic groups.

The salicylate polymer can be calculated, based on elemental analysis data, the analyses discussed above, and the assumption that polysulfanilamide 13 is a homopolymer, to consist of 75–80 mer % 5-azo-leashed salicylate groups, 10–15 mer % *p*-hydroxy-*N*-vinylbenzenesulfonamide groups, and 5–10 mer % sodium *p-trans*-diazotato-*N*-vinylbenzenesulfonamide groups. Trace mer impurities known to be present are 3-azo-leashed salicylate groups (~1 mer %), residual *p*-acetamido-*N*-vinylbenzenesulfonamide groups (~1 mer %), and triazine links (≤0.5 mer %).[39] Other mer groups, including most notably residual backbone vinylamine and amidine residues, may also be present in a given sample depending on the effort made to exclude their presence at the appropriate step of the synthetic sequence.

In Vitro Metabolism. In bacterial whole-cell experiments, polymeric salicylate 7 and the previously investigated[28] polymeric analogue of FD & C Yellow No. 6 (as an azo reduction control) were reduced about 50 and 20%, respectively, in 6 h in the absence of benzylviologen. With this redox mediator present, both polymers were completely reduced in less than 2 h. Anaerobic cell-free extracts (CFE) reduced 7 at rates from 0.35 to 1.09 μequiv of azo bond reduced h^{-1} (mL of CFE)$^{-1}$, depending on the concentrations of CFE and benzylviologen used. These results indicate that the kinetics and mechanism of the bacterial azo bond cleavage of 7 are essentially identical with those of polymeric azo compounds studied previously.[26,40]

In Vivo Metabolism. The whole-cell data were employed to estimate an in vivo reduction rate of 1 μequiv of azo bond reduced h^{-1} (mL of cecal contents)$^{-1}$ for the rat. Assuming an experimental animal to have 3 mL of cecal contents and the maximum residence time to be 10 h (corresponds to 30 μequiv of azo bond reduction capability), an oral dosage of 37 μequiv of azo bond was selected to be certain of an excess.[41]

Table I presents peak serum concentrations and urinary and fecal recoveries of 5-ASA (3) and metabolites after oral administration of SASP (1) and polymer 7 to rats. Urinary 5-ASA and metabolites were significantly higher in the polymer group than in the SASP group (8.0 vs. 3.7%). Fecal recovery and total recovery were not, however, significantly different. The peak serum concentration of the polymer group (2.5 nmol/mL) was statistically lower than the SASP-dosed group (5.6 nmol/mL). Although there were variations among individual animals receiving both compounds, similar trends were: (a) a large percentage of

(39) Polymeric diazonium salt 14 reacts with polysulfanilamide 13 to produce insolubilizing triazine cross-links.[25] This reaction can (and sometimes does) result in polymer precipitation during the diazotization step.

P—NH—N=N—P

Although it is possible to avoid precipitation by efficient mixing of the polymer and nitrite solutions (all sulfanilamide is completely diazotized before triazine formation can take place), some minute amount of triazine linkage is probably present in the final product. From statistical considerations, the amount of triazine cross-link cannot be greater than 0.5 mer % or a soluble product would not be obtained. See P. J. Flory, "Principles of Polymer Chemistry", Cornell University Press, Ithaca, NY, 1953, p 359.

(40) (a) P. L. Dubin and K. L. Wright, *Xenobiotica*, **5**, 563 (1975). (b) J. P. Brown, *Abstr. Annu. Meet. Am. Soc. Microbiol.*, 123 (1976).

(41) This dosage (0.11 mequiv of azo bond/kg) is close to the daily human therapeutic dose of sulfasalazine (0.14 mmol/kg).

Table III. Guinea Pig Quantitative Histological Evaluation Results[a]

group[b]	no. of observations	mean score ± SEM[c]	Student's t value	significance vs. control
control	16	3.25 ± 0.17		
SASP	12	2.68 ± 0.38	1.38	$p > 0.10$
SP	18	3.11 ± 0.25	0.46	$p > 0.10$
5-ASA	15	2.40 ± 0.28	2.57	$0.01 < p < 0.02$
polymer 7	15	2.16 ± 0.28	3.34	$0.001 < p < 0.01$

[a] Experiment based on 60 μmol of SASP, 60 μmol of SP, 65 μmol of 5-ASA, and 56 μequiv of azo bond of polymer 7. [b] All groups contained 20 animals, except the 5-ASA group which contained 18. [c] Average of two independent reviewers.

total urinary excretion of 5-ASA and metabolites (39 to 83%) took place between 12 and 24 h; (b) the majority of total fecal excretion of 5-ASA and metabolites (69 to 98%) occurred between 8 and 36 h; and (c) serum concentration peaked at the 12-h point.

Guinea Pig Studies.[42] Carrageenan-induced colitis in guinea pigs simulates the lesions of acute human ulcerative colitis[43] and is reported to respond to SASP treatment in some studies but not in others.[31] Physical findings and necropsy results for a comparison of SASP (1), SP (2), 5-ASA (3), and polymer 7 in this animal model are presented in Table II. The control group developed cecal ulcerations with mortality, weight loss, diarrhea, and necropsy findings comparable to those reported for previous studies.[30,31,44] This group's fluid consumption remained relatively stable at 60 mL/day (range 57–90 mL/day). SASP administration did not substantially alter disease development and afforded results similar to the control group. Mean fluid consumption for this group was 65 mL/day (range 48–57 mL/day). SP afforded results similar to the control group, although the mean weight loss for this group (17 g) was somewhat lower than the control group (29 g). Mean fluid consumptions for the polymer-dosed and 5-ASA-dosed groups were 56 and 61 mL/day, respectively. Both polymer 7 and 5-ASA produced reductions of gross abnormalities relative to the control group. Both groups also exhibited less severe disease manifestations than the control group, although gross pathology findings were graded positive regardless of disease severity.

Results of a quantitative histological evaluation of the guinea pigs is given in Table III. Histopathology results were scored virtually identically by the two reviewers. Polymer 7 afforded the lowest mean score and was significantly different than the control group. Disease severity was also significantly reduced by 5-ASA. SASP produced a statistically insignificant reduction in score relative to the control group. SP produced no effect.

Discussion

In vitro studies with anaerobic rat cecal bacteria have shown polymer 7 to undergo azo bond cleavage by a reductive process analogous to that previously described for similar materials.[26,40] A pharmacokinetic comparison of orally administered polymer and SASP has demonstrated comparable deliveries of 5-ASA and metabolites to the rat lower bowel. The mean peak serum concentration of 5-ASA and metabolites was significantly lower in the polymer-treated group than in the SASP-treated group, while the mean urinary excretion of 5-ASA and metabolites was significantly higher in the polymer-treated rats.[45] Pieniaszek and Bates have reported that 5-ASA is subject to both capacity-limited presystemic and systemic acetylation in orally dosed rats.[46] If 5-ASA is released more slowly in the lower bowel from the polymer than from SASP, 5-ASA acetylation would be enhanced. Increased 5-ASA acetylation would produce higher urinary excretions of *N*-Ac-ASA and lower serum levels of 5-ASA and metabolites. This hypothesis remains unproven, since *N*-Ac-ASA was not independently quantitated by the modified Hansson method employed for metabolite assay.

Lower mean percentages for the fecal excretion of 5-ASA and metabolites were observed in the present study (11.0% after 96 h for the SASP group, and 12.3% after 96 h for the polymer group) than was reported for an earlier study of oral SASP administration to rats (about 28% of a 250 μmol dose after 72 h[47]). There are a number of important differences between the animals and metabolite quantitation methods employed in the two studies. The rats of this study were not fasted, while those of the previous study were fasted for 24 h pre- and postdosing. This will produce differences in gastrointestinal transit time and in intestinal microflora activity. A modified Brattan–Marshall assay was used in the earlier study for metabolite determination. This study employed a spectrofluorometric assay in which fluorescence quenching lowered recoveries. No attempt to correct for this phenomenon was made, since the goal of this study was to obtain relative quantitative results (not absolute values) for polymer 7 and SASP under identical experimental conditions.[48]

Salicylate polymer 7 and 5-ASA decreased the carrageenan-induced inflammatory response of the guinea pig ulcerative colitis model based on the quantitative histopathological results. SASP also reduced the inflammatory response, but the result was not statistically significant. SP did not affect the inflammatory response. Guinea pig mortality was not reduced by any test compound. Since secondary factors unaffected by treatment, such as coliform bacteria,[30] may contribute to death, mortality is not used to evaluate the potential effectiveness of drugs for human ulcerative colitis with this animal model. Gross and microscopic manifestations of inflammation and ulceration are the primary evaluation cirteria.[31] These criteria indicate polymer 7 to be the most potentially effective of the compounds tested.

(42) A preliminary report of these studies was presented at the International Symposium on Inflammatory Bowel Disease, Jerusalem, Israel, Sept 7–9, 1981.

(43) (a) J. Watt and R. Marcus, *J. Pharm. Pharmacol.*, **21**, 1877 (1969); (b) *Gut*, **12**, 164 (1971); (c) *ibid.*, **14**, 506 (1973).

(44) A. B. Onderdonk, T. J. Louie, F. P. Tally, and J. G. Bartlett, *J. Antimicrob. Chemother.*, **5**, 201 (1979).

(45) 5-ASA serum concentration has been correlated with clinical efficacy,[11] but 5-ASA colonic actions are probably responsible for therapeutic effects.[14–16]

(46) H. J. Pieniaszek and T. R. Bates, *J. Pharm. Sci.*, **68**, 1323 (1979).

(47) H. J. Pieniaszek and T. R. Bates, *J. Pharmacol. Exp. Ther.*, **198**, 240 (1976).

(48) Both groups of rats in this study afforded lower percentages for fecal excretion and total recovery of 5-ASA and metabolites than have been reported for orally administered SASP in man.[6] These differences are probably caused by the different transit times of the two species. See P. A. M. Van Hees, J. H. M. Tuinte, J. M. Van Rossum, and J. H. M. Van Tongeren, *Gut*, **20**, 300 (1979).

After completion of our studies, preliminary reports appeared indicating possible effectiveness in ulcerative colitis patients of two other azo-linked derivatives of 5-ASA, sodium azodisalicylate[49] and salicylazobenzoic acid,[50] administered as retention enemas. More recently a sustained-release preparation of 5-ASA has been described[51] which delivers 5-ASA to both the small and large intestines during its transit through the gastrointestinal tract.

In summary, comparable total release of 5-ASA and metabolites has been demonstrated in rats for polymer 7 and SASP, and polymer 7 has been shown to be more effective than SASP in reducing the inflammation of the guinea pig ulcerative colitis model. Since SASP metabolism proceeds by the same reductive pathway in both rats and man,[7] polymer 7 may provide a new oral dosage form for the site-specific delivery of therapeutic levels of 5-ASA to the lower human bowel, which will eliminate the adverse side effects currently limiting the SASP therapy of inflammatory bowel disease.[52]

Acknowledgment. The authors gratefully acknowledge M. Kronstadt and R. Phillips for polymer analyses and for providing previously unpublished results; P. Brock, L. Bunes, D. Dawson, S. Ng, and S. Swanson for helpful chemical discussions and for providing model analytical compounds; R. Cisneros, F. Enderlin, and C. Smith for technical assistance with the biological studies; and K. Isselbacher for discussions concerning the therapeutic mechanism of SASP.

Registry No. 13, 86260-27-7.

(49) (a) C. P. Willoughly, J. K. Aronson, H. Agback, N. O. Bodin, E. Anderson, and S. C. Truelove, *Gut*, **22**, A431 (1981); (b) D. P. Jewell and S. C. Truelove, *Lancet*, **2**, 1168 (1981).
(50) A. Bartalsky, *Lancet*, **1**, 960 (1982).
(51) S. N. Rasmussen, S. Bondesen, D. F. Hvidberg, S. H. Hansen, V. Binder, S. Halskov, and H. Flachs, *Gastroenterology*, **83**, 1062 (1982).
(52) P. Goldman, *Gastroenterology*, **83**, 1138 (1982).

Notes

Heterocyclic Oxyacetic Acid Diuretics: Indazole, Benzisothiazole, and Benzisothiazole 1,1-Dioxide Analogues of [[7-Chloro-3-(2-fluorophenyl)-1,2-benzisoxazol-6-yl]oxy]acetic Acid

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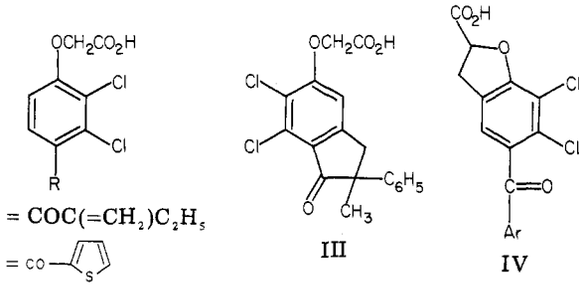
The indazole, benzisothiazole, and benzisothiazole 1,1-dioxide analogues of [[7-chloro-3-(2-fluorophenyl)-1,2-benzisoxazol-6-yl]oxy]acetic acid were synthesized and tested for diuretic activity in saline-loaded mice. Each analogue was found to be less active than the parent benzisoxazole: the diuretic activity followed the order O > S > N = SO_2 in regard to the heteroatom in the 1-position of the ring.

We recently reported[1] a series of [(3-aryl-1,2-benzisoxazol-6-yl)oxy]acetic acids with potent diuretic activity in mice and dogs. These compounds are new members of the family of phenoxyacetic acid diuretics (Chart I), a family that has grown from ethacrynic acid (I) and tienilic acid (II) to include indacrinone (III), as well as the 5-acylbenzofuran-2-carboxylic acids (IV) and the [4-(arylsulfonyl)phenoxy]acetic acids (V). These compounds are characterized by their uricosuric, as well as diuretic, properties: the spectrum of activity ranges from loop diuretics that cause uric acid retention (I), to uricosuric loop diuretics (III), to uricosuric diuretics with a low-ceiling profile (II). The [(3-aryl-1,2-benzisoxazol-6-yl)oxy]acetic acids occupy their own niche among the phenoxyacetic acid diuretics with a unique profile of diuretic and uricosuric activity.

Among the heterocyclic oxyacetic acids in our previous publication, diuretic activity was maximal in compounds **1a–c** in which the 3-aryl substituent was 2-chloro- or (better) 2-fluorophenyl and the benzisoxazole ring was substituted in the 7-position with either chlorine (**1a,b**) or bromine (**1c**). These compounds also showed good intravenous activity in the dog. Because **1c** (HP 522) demonstrated good diuretic activity, as well as mild uricosuria, with no rebound after chronic oral dosing in chimpanzees,

Chart I. Structures of Some Phenoxyacetic Acid Diuretics



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(1) Shutske, G. M.; Setescak, L. L.; Allen, R. C.; Davis L.; Effland, R. C.; Ranbom, K.; Kitzen, J. M.; Wilker, J. C.; Novick, Jr., W. J. *J. Med. Chem.* **1982**, *25*, 36.

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