Notes

Benzisoxazolones: Antimicrobial and Antileukemic Activity

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An unusual acid-mediated rearrangement of *o*-nitrostyrene oxide afforded 1-(hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one which exhibited broad-spectrum antimicrobial and cytotoxic activity. A number of analogues were prepared by employing a modified zinc-reduction procedure on *o*-nitrobenzoate. Several of these analogues exhibited interesting antipseudomonal activity in agar and broth but were ineffective in vivo.

During the course of examining the utility of various photolabile prodrugs,[1] we uncovered an interesting, acid-mediated rearrangement of *o*-nitrostyrene oxide (**1**) to 1-(hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one [**2**, (Scheme I)].[2] Routine biological screening revealed Gram-positive and Gram-negative antibacterial activity as well as modest cytotoxic activity against L1210 leukemia cells in suspension. Of particular interest was the finding of antipseudomonas activity. However, in the process of determining minimum inhibitory concentrations against various organisms in broth, we noted variable results that we attributed to instability of **2** in protic media. Therefore, in view of the interesting activity, absence of literature precedent for antimicrobial activity in this structural class, and relative instability, we initiated efforts to prepare and evaluate analogues of this benzisoxazolone.

Chemistry. Benzisoxazol-3(1*H*)-one (**3**) was originally prepared by zinc reduction of *o*-nitrobenzoates.[3] We therefore employed this approach with modified conditions[2] as an alternate synthesis of **2** as well as to generate analogues. Several *N*-acyl analogues (**4**) were prepared from **3** by using standard conditions. Similarly, various *O*-acyl derivatives (**5**) were synthesized from the (hydroxymethyl)benzisoxazolone **2** by employing the appropriate anhydride, acid chloride, or isocyanate.

In addition we found that analogues of **4** could be secured in a one-pot synthesis from methyl-*o*-nitrobenzoate in a similar fashion to **2** by substituting methyl isocyanate or methanesulfonyl chloride for formaldehyde (Scheme II). Interestingly, in an attempt to methylate the hydroxy group of **2** we produced only the *N*-methyl compound (Scheme II) resulting from the presumed silver oxide catalyzed loss of formaldehyde. However, the desired *O*-methyl was prepared as shown in Scheme II from *o*-nitrobenzoate and $Zn/CH_2O/CH_3OH$, albeit in very poor yield.

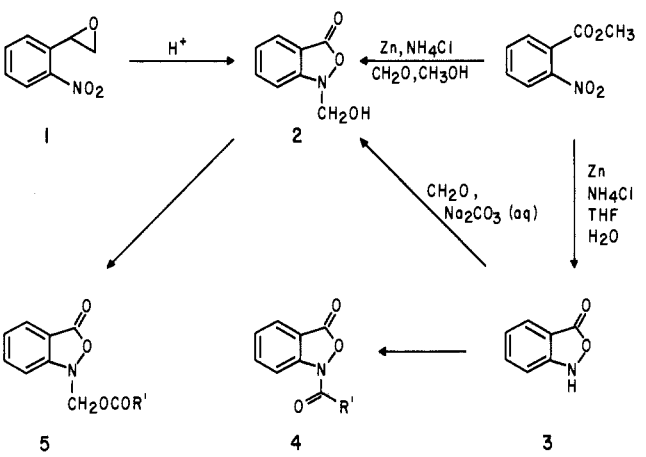
Biology. Preliminary antimicrobial analysis of the benzisoxazolones with an agar diffusion assay is tabulated in Table I. As is evident from the table, there is broad-spectrum antibacterial activity as well as antifungal activity (*Penicillium oxalicum*). In addition there is listed in Table I the cytotoxic activity of these analogues against L1210 cells in suspension expressed as inhibitory doses in microgram/milliliter.

The data indicate the following trends in structure–activity relationships: (1) analogues bearing *N*-(alkylacyl)oxy substituents, represented by structure **5**, exhibit the best activity, (2) analogues bearing long-chain, lipophilic groups have minimal activity, and (3) simple *N*-alkyl or *N*-sulfonyl groups abolish activity. We speculate that activity is dependent on a reactive aminal grouping coupled with, or in addition to, a reactive carbonyl group. This is further supported by the observation of reduced activity in several analogues with alkoxy (i.e., electron-donating) substitutents on the aromatic ring.

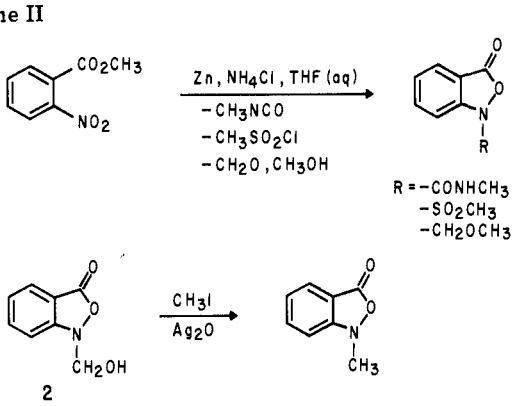
Several of the lead candidates were evaluated in a microplate broth dilution assay (Table II). Unfortunately none of the analogues exhibited superior activity over the parent structure, **2**. Indeed, several were less effective against some organisms.

In vivo evaluation involved *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* infections in mice. Benzisoxazolone **2** was evaluated subcutaneously at a maximum

Scheme I



Scheme II



(1) Voorhees, J. J.; Wierenga, W. U.S. Patent 4 302 456 (7), 1981.
(2) Wierenga, W.; Harrison, A. W.; Evans, B. R.; Chidester, C. *J. Org. Chem.* **1984**, *49*, 438.
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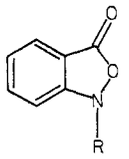
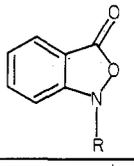
Table I. Antimicrobial and Antileukemic Activity of Benzisoxazolones



R	organism[a]												L1210 antileukemic,[b] μg/mL	
	B.s.	*K.p.*	*M.l.*	*E.s.*	*P.v.*	*P.c.*	*P.f.*	*S.a.*	*S.s.*	*M.a.*	*P.o.*	*S.p.*	ID_{50}	ID_{90}
$COCH_3$ (6)	24	20	18	26	23	38	31		20	22	49	21	3.4	8.7
$CONHCH_3$ (7)		16			19	24				22			4.9	14
$CONH(CH_2)_4CH_2I$ (11)						inactive							3.7	8.6
SO_2CH_3 (8)						31							4.3	8.7
CH_3 (10)						inactive							ND	ND
CH_2OCH_3 (9)						inactive							ND	ND
CH_2OH (3)	22	26	27	25	30	30	35	15	26	26	41	16	3.1	8.0
CH_2OCOCH_3 (12)	24	24	24	26	25	32	32	15	22	21	43	17	5.1	19
$CH_2OCOCH(CH_3)_2$ (13)	17	22	22	17	24	22	33	17	23	21	29		5.3	20
$CH_2OCOC(CH_3)_3$ (14)	18	21	18	17	18	20	30	18	22	20	31		4.2	16
$CH_2OCO(CH_2)_4CH_2I$ (15)	15				15	17	23			16	26		19	43
$CH_2OCO(CH_2)_{14}CH_3$ (16)						inactive							ND	ND
$CH_2OCOC_6H_4CH_3(p)$ (17)	20	21		18	22	22	20		17	17	24		4.6	17
$CH_2OCOCH_2OC_6H_5$ (18)	20	22	19	18	21	19	31	15	20	19	24		12	38
$CH_2OCONHCH_3$ (19)	16	23	33	30	32	39	33		28	20	46		3.5	8.6

[a] Diameter of zone of inhibition against organism indicated produced by a 13-mm Schleischer & Schuell cotton disk dipped in a 1 mg/mL acetone solution followed by drying and application to agar surface. A standard antibiotic was employed to validate the assays (data ± 3 mm between assays). *B.s.*, *Bacillus subtilis*; *K.p.*, *Klebsiella pneumoniae*; *M.l.*, *Micrococcus luteus*; *E.c.*, *Escherichia coli*; *P.v.*, *Proteus vulgaris*; *P.a.*, *Pseudomonas aeruginosa*; *P.f.*, *Pseudomonus fluorescens*; *S.a.*, *Staphylococcus aureus*; *S.s.*, *Salmonella schottmuelleri*; *M.a.*, *Mycobacterium avium*; *P.o.*, *Penicillium oxalicum*; *S.p.*, *Saccharomyces pastorianus*. [b] Li, L. H.; Kuentzel, S. L.; Murch, L. L.; Pschigoda, L. M.; Krueger, W. C. *Cancer Res.* 1979, *39*, 4816-4822.

Table II. Antimicrobial Activity for Selected Benzisoxazolone Analogues



organism	MIC,[b] μg/mL			
	2	**12**	**19**	**6**
Staphylococcus aureus UC76	62.5	>250	62.5	62.5
Streptococcus faecalis UC694	250	>250	ND[a]	250
Escherichia coli UC45	15.6	125	31.2	31.2
Klebsiella pneumoniae UC58	31.2	62.5	62.5	62.5
Pseudomonas aeruginosa UC95	7.8	31.2	15.6	15.6
Proteus vulgaris UC93	3.9	15.6	15.6	15.6
Proteus mirabilis UC6671	15.6	62.5	31.2	125
Shigella flexneri UC143	15.6	62.5	31.2	125
Salmonella typhi UC215	7.8	62.5	15.6	31.2
Serratia marcescens UC131	2.0	15.6	3.9	31.2
Salmonella schottmuelleri UC126	31.2	62.5	31.2	15.6
Providencia stuartii UC6570	31.2	125	62.5	62.5

[a] Not determined. [b] See Experimental Section.

tolerated dose of 150 mg/kg and determined to be ineffective against *K. pneumoniae*. In addition benzisoxazolone **19** (R = $CH_2OCONHCH_3$) and **2** were tested ip at 200 mg/kg and exhibited no activity against *P. aeruginosa*.

Followup in vivo evaluation of the in vitro L1210 activity was performed on these same two compounds against P388 leukemia (ip).[4] They were administered ip on a Q4DX3 schedule (days 1, 5, 9) posttumor inoculation. No increase in life span was evident up to a maximum tolerated dose of 200 mg/kg for **2** and 400 mg/kg for benzisoxazolone **19** (R = $CH_2OCONHCH_3$).

Experimental Section

1-[(Acetyloxy)methyl]-2,1-benzisoxazol-3(1*H*)-one (12). One milliliter of acetic anhydride was cooled under N_2 in an ice bath. To this was added **2** (0.14 g, 0.84 mmol) followed by a small spatula tip of sodium acetate or a drop of pyridine. The reaction mixture was stirred and allowed to warm to room temperature overnight. Five milliliters of ethanol or water was then added and after 10 min the reaction mixture was diluted with 50 mL of ethyl acetate, washed once with water and once with brine, filtered through sodium sulfate, and evaporated to dryness, giving a white solid (0.18 g). This was transferred to two 2000-μm silica gel plates and developed once with 40% ethyl acetate/hexane. The R_f 0.6 band was isolated as a white solid (0.124 g, 71% yield). An analytical sample was recrystallized from diethyl ether to give colorless crystals (mp 121–122.5 °C): NMR ($CDCl_3Me_4Si$) 7.9–7.2 ppm (m, 4 H, aromatic H), 5.8 (s, 2 H, CH_2), 1.9 (s, 3 H, methyl); MS, *m/e* 207.0541 (M^+). Anal. ($C_{10}H_9NO_4$) C, H, N.

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one 2-Methylpropanoate (13). Following the procedure and workup used for **12** on an 0.33-mmol scale, the organic layer was dried over sodium sulfate and concentrated to a colorless oil (0.074 g, 89% yield). An analytical sample was crystallized from diethyl ether (−78 °C) to give a white solid (mp 49.5–50.5 °C): NMR ($CDCl_3/Me_4Si$) 7.90–7.22 ppm (m, 4 H, aromatic H), 5.81 (s, 1 H, CH_2O), 2.47–2.29 (quintet, 1 H, J = 7.4 Hz, dimethyl CH), 0.91 (6 H, d, J = 7 Hz, dimethyl H); IR (CH_2Cl_2) 1780 cm^{-1} (s, C=O); MS, *m/e* 235.0837 (M^+). Anal. ($C_{12}H_{13}NO_4$) C, H, N.

2-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one 2,2-Dimethylpropanoate (14). Following the procedure and workup used for **12** on an 0.5-mmol scale, the organic layer was then dried over sodium sulfate and concentrated to a yellow oil (0.13 g). This oil was transferred to two 2000-μm silica gel plates and developed once with 25% ethyl acetate/hexane. The R_f 0.5 band was isolated as a colorless oil (0.078 g, 59% yield). An analytical sample was crystallized from diethyl ether (−78 °C) to give a white solid (mp

(4) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbot, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1.
(5) Lewis, C.; Clapp, H. W. *Antibiot. Chemother.* **1961**, *11*, 127.

36–37 °C): NMR ($CDCl_3Me_4Si$) 7.98–7.23 ppm (m, 4 H, aromatic H), 5.87 (s, 2 H, methylene H), 0.95 (s, 9 H, methyl H); IR (CH_2Cl_2) 1780 cm^{-1} (s, C=O), 1720 (s, C=O); MS, *m/e* 249.1008 (M^+). Anal. ($C_{13}H_{15}NO_4$) C, H, N; N calcd, 5.62; found, 6.27.

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one 4-Methylbenzoate (17). Methylene chloride (1 mL), triethylamine (0.094 mL, 6.7 mmol), and **2** (0.053 g, 0.32 mmol) were cooled under N_2 in a dry ice–2-propanol bath. To this mixture was added *p*-toluoyl chloride (0.043 mL, 0.32 mmol) and the resultant mixture was allowed to slowly warm to near room temperature over a period of 5 h. The mixture was then diluted with 50 mL of ethyl acetate and washed successively with water (30 mL), 1.2 N aqueous HCl (30 mL), water (30 mL), and aqueous sodium carbonate (30 mL), dried over sodium sulfate, and concentrated to a white solid (0.088 g). This was transferred to two 200-μm silica gel plates and developed once with 35% ethyl acetate/hexane. The R_f 0.6 band was isolated as a colorless oil, which slowly crystallized (0.065 g, 72% yield): NMR ($CDCl_3/Me_4Si$) 7.97–7.12 ppm (m, 8 H, aromatic H), 6.05 (s, 2 H, methylene H), 2.35 (s, 3 H, methyl H).

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one Hexadecanoate (16). The reaction was run and worked up as described for **17**, substituting palmitoyl chloride (0.34 mmol). The R_f 0.4 band (15% ethyl acetate/hexane) was isolated as a white solid (0.06 g, mp 63–64.5 °C, 44% yield): NMR ($CDCl_3/Me_4Si$) 8.00–7.2 ppm (m, 4 H, aromatic H), 5.85 (s, 2 H, *N*-methylene H), 2.33–2.03 (m, 2 H, methylene H α to carbonyl), 1.67–0.87 (m, 29 H, remaining methylene and methyl H); IR (CH_2Cl_2) 1790 cm^{-1} (s, C=O); MS, *m/e* 148 [$M^+ - CO_2(CH_2)_{14}CH_3$]. Anal. ($C_{24}H_{37}NO_4$) C, H, N.

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one Phenoxyacetate (18). The reaction was run and worked up as for **17**, substituting phenoxyacetyl chloride (0.33 mmol). The R_f 0.5 band was isolated as a white solid (0.04 g, 40% yield). Recrystallization from diethyl ether/ethyl acetate at −78 °C gave white crystals (mp 101–102.5 °C): NMR ($CDCl_3/Me_4Si$) 7.97–6.6 ppm (m, 9 H, aromatic H), 5.93 (s, 2 H, *N*-methylene H), 4.5 (s, 2 H, phenoxy-methylene H); MS, *m/e* 299.0788 (M^+). Anal. ($C_{16}H_{13}N_1O_5$) C, H, N.

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one Methylcarbamate (19). Methyl isocyanate (4 mL) and **2** (0.15 g, 0.9 mmol) were cooled under N_2 in a dry ice–2-propanol bath, and then triethylamine (0.15 mL 1.1 mmol) was added to the reaction mixture, which was then allowed to warm to near room temperature. The mixture was then diluted with ethyl acetate and shaken with water (4 × 30 mL) and brine (30 mL). The organic layer was then dried over sodium sulfate and concentrated to a colorless oil, which slowly crystallized (0.237 g). This was transferred to three 2000-μm silica gel plates and developed once with 50% ethyl acetate/hexane. The R_f 0.4 band was isolated as light brown crystals (0.17 g), which were dissolved in diethyl ether/ethyl acetate, treated with activated charcoal, and filtered. The filtrate was concentrated to dryness, giving colorless crystals (0.16 g, 79% yield). An analytical sample was crystallized from diethyl ether to give colorless crystals (mp 99.5–100 °C): NMR ($CDCl_3/Me_4Si$) 7.88–7.18 ppm (m, 4 H, aromatic H), 5.78 (s, 2 H, methylene H), 5.4 (m, 1 H, NH), 2.67 (d, J = 4.5 Hz, 3 H, methyl H); MS, *m/e* 222 (M^+), 148 ($M^+ - CO_2N(H)CH_3$), 135 ($M^+ - CH_2O_2CNHCH_3$). Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

1-(Hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one (5-Iodopentyl)carbonate (15). Following the procedure and workup for **19** on an 0.4-mmol scale, the R_f 0.4 band (35% ethyl acetate/hexane) was isolated as an off-white solid (0.09 g, 58% yield). Recrystallization from diethyl ether gave colorless crystals (mp 104–105 °C): NMR ($CDCl_3/Me_4Si$) 7.97–7.25 ppm (m, 4 H, aromatic H), 5.82 (s, 2 H, ring *N*-methylene H), 5.0 (s, 1 H, NH), 3.62–2.83 (m, 4 H, methylenes α to NH and I), 2.03–1.00 (m, 6 H, remaining methylene H); MS, *m/e* 404 (M^+), 148 [($M^+ - CO_2$ iodopentyl)], 135 [2,1-benzisoxazol-3(1*H*)-one].

***N*-Methyl-3-oxo-3,1-benzisoxazole-1(3*H*)-carboxamide (7).** A mixture of zinc (0.17 g, 2.6 mmol), ammonium chloride (0.4 g, 7.5 mmol), and methyl *o*-nitrobenzoate was stirred vigorously in a mixture of THF and H_2O (5 mL each). After 5 min, methyl isocyanate (0.07 mL, 1.1 mmol) was added to the mixture. At 1.5 and 5 h, another 0.07 mL methyl isocyanate was added (for a total of 0.21 mL added) and then the reaction mixture was stored (without stirring) in a freezer for 72 h. The mixture was then diluted with ethyl acetate, washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4, and concentrated to give a yellow solid (0.186 g). This was dissolved in diethyl ether, treated with decolorizing carbon, filtered, and concentrated to a white solid (0.152 g), which was transferred to two 2000-μm silica gel plates and developed once with 35% ethyl acetate/hexane. The R_f 0.5 band was isolated as a white solid (0.104 g, 49% yield, mp 139–140 °C): NMR (acetone-d_6/Me_4Si) 8.08–7.25 ppm (m, 4 H, aromatic H), 2.97–2.9 (m, 4 H, NH and methyl H); MS, *m/e* 192.0539 (M^+).

1-(Methylsulfonyl)-2,1-benzisoxazol-3(1*H*)-one (8). Triethylamine (0.2 mL, 1.4 mmol), benzisoxazol-3(1*H*)-one[2] 3 (0.19 g, 1.4 mmol), 4-(dimethylamino)pyridine (0.02 g, 0.16 mmol), and 5 mL of methylene chloride were cooled with stirring under N_2 in an ice bath. To this cooled mixture was added methanesulfonyl chloride (0.13 mL, 1.7 mmol). After 2 h, 5 mL of water was added to the reaction mixture, which was then diluted with ethyl acetate (60 mL) and shaken with water (30 mL), aqueous $NaHCO_3$, and brine. The organic layer was then dried over sodium sulfate and the solvent evaporated to give a dark oil (0.237 g). This oil was transferred to two silica gel chromatography plates (2000 μm) developed once with 50% ethyl acetate/hexane. The R_f 0.7 band was isolated as brownish crystals (0.17 g). These crystals were dissolved in diethyl ether, treated with decolorizing carbon, and filtered, and the solvent was evaporated to give a light solid (0.083 g, mp 94.5–95.5 °C, 28% yield): NMR (acetone-d_6/Me_4Si) 8.2–7.55 ppm (m, 4 H, aromatic H), 3.23 (s, 3 H, methyl); MS, *m/e* 213.0094 (M^+). Anal. ($C_8H_7NO_4S$) C, H, N, S.

***N*-(5-Iodopentyl)-3-oxo-2,1-benzisoxazole-1(3*H*)-carboxamide (11).** Methylene chloride (3 mL) and 3 (0.22 g, 1.6 mmol) were cooled under N_2 in a dry ice–2-propanol bath. To this cooled mixture were added triethylamine (0.25 mL, 1.8 mmol) and 5-iodopentyl isocyanate (0.257 mL, 1.6 mmol). After 3 h the still cold reaction mixture was mixed with water (3 mL) and then diluted wih ethyl acetate (50 mL). The organic layer was then shaken successively with water (4 × 30 mL) and brine (30 mL), dried over sodium sulfate, and concentrated to a white solid (0.568 g). This was recrystallized from diethyl ether to give fluffy, white crystals (mp 109.5–110.5 °C): NMR ($CDCl_3/Me_4Si$) 7.93–7.22 ppm (m, 4 H, aromatic H), 5.90 (s, 1 H, NH), 3.54–3.11 (m, 4 H, methylene H α to N and I), 2.05–1.3 (m, 6 H, remaining methylene H); MS, *m/e* 374 (M^+), 135 [2,1-benzisoxazol-3(1*H*)-one].

1-Acetyl-2,1-benzisoxazol-3(1*H*)-one (6). 2,1-Benzisoxazolone (3; 0.043 g, 0.3 mmol) and a small spatula tip of sodium acetate were cooled under nitrogen in an ice bath. To this was added 0.5 mL of acetic anhydride. The stirred mixture was allowed to warm to room temperature overnight and then was evaporated to dryness, giving a brown solid. This was transferred to a 2000-μm silica gel plate and developed once with 30% ethyl acetate/hexane. The R_f 0.7 band was isolated as a pale brown solid (0.038 g, 67% yield). An analytical sample was recrystallized from diethyl ether to give pale brown crystals (mp 119–120 °C): NMR ($CDCl_3/Me_4Si$) 8.18–7.21 ppm (m, 4 H aromatic H), 2.47 (s, 3 H methyl H); IR (CH_2Cl_2) 1780 cm^{-1} (s, ring C=O), 1700 (s, acetyl C=O); MS, *m/e* 177.0439 (M^+). Anal. ($C_9H_7NO_3$) C, H, N.

1-Methyl-2,1-benzisoxazol-3(1*H*)-one (10). Methyl iodide (0.12 g, 0.84 mmol, 0.05 mL) was added at room temperature to a heterogeneous mixture of silver oxide (0.13 g, 0.57 mmol) and 1-(hydroxymethyl)-2,1-benzisoxazol-3(1*H*)-one (**2**; 0.046 g, 0.28 mmol) in DMF. After overnight stirring, the mixture was filtered, diluted with 20 mL of EtOAc, and washed with water (1 × 10 mL), aqueous Na_2CO_3 (2 × 10 mL), and brine (1 × 20 mL), dried (Na_2SO_4), and concentrated to a light brown oil (0.035 g). This oil was transferred to a 2000-μm silica gel plate and developed once with 30% EtOAc/hexane. The R_f 0.5 band was isolated as a yellow oil (0.023 g, 55% yield): NMR ($CDCl_3/Me_4Si$): 7.92–7.03 (m, 4 H aromatic H), 3.37 (s, 3 H, methyl H).

1-(Methoxymethyl)-2,1-benzisoxazol-3(1*H*)-one (9). Zinc dust (0.2 g, 3 mmol) was added to a heterogeneous mixture of ammonium chloride (0.4 g, 7 mmol) and methyl *o*-nitrobenzoate in 10 mL of methanol. To this mixture were then added Na_2CO_3 (0.06 g, 0.56 mmol) and 37% aqueous formaldehyde (1.1 mL). After 2.5 h of vigorous stirring the run mixture was filtered, diluted with diethyl ether, and shaken with aqueous Na_2CO_3 (2 × 30 mL), water (30 mL), and brine (30 mL), dried over Na_2SO_4, and concentrated in vacuo to an off-white solid (0.09 g). This was

transferred to two 2000-μm silica gel chromatography plates and developed once with 35% EtOAc/hexane. The R_f 0.6 band was isolated (0.003 g): NMR ($CDCl_3/Me_4Si$) 7.45–7.0 (m, 4 H aromatic H), 5.02 (s, 2 H, methylene), 3.8 (s, 3 H methoxy H); IR (CH_2Cl_2) 1780 cm^{-1} (C=O); MS, 179.0578 (parent).

Antimicrobial Activity: Minimum Inhibitory Concentration. Minimum inhibitory concentration (MIC) for various bacteria was determined by a microplate broth dilution technique. Serial twofold dilutions of the antibiotics were prepared in 50 μL of modified brain heart infusion broth medium (reference) in the wells of a microplate. Each well was then inoculated with 50 μL of standardized cell suspension to yield a final concentration of $\sim 10^5$ viable cells per milliliter of drug-supplemented medium. The microplates were incubated at 37 °C for 20 h, and the MIC was read as the lowest concentration of drug that inhibited the visible growth of the organism.

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Registry No. 1, 39830-70-1; 2, 88057-16-3; 3, 31499-90-8; 6, 33047-12-0; 7, 23091-67-0; 8, 90720-08-4; 9, 90720-09-5; 10, 90720-10-8; 11, 90720-11-9; 12, 90720-12-0; 13, 90720-13-1; 14, 90720-14-2; 15, 90720-15-3; 16, 90720-16-4; 17, 90720-17-5; 18, 90720-18-6; 19, 90720-19-7.

Synthesis and Pharmacological Evaluation of Indanpropionic Acids as Uterine Relaxants[1]

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The $PGF_{2\alpha}$ antagonist 5,6-bis(benzyloxy)-1-oxo-2-propyl-2-indanpropionic acid (1) had previously been shown to provide significant protection against the abortifacient actions of $PGF_{2\alpha}$ in mice. To explore further structural concepts in drug design employed for the development of 1, several mono(benzyloxy) ketones (3–10) and alcohols (11–15) as well as a diacid (22) were prepared. None of these structural modifications resulted in compounds of greater superiority to 1 as uterine relaxants and 22 was void of any antagonistic properties, suggesting that the original rationale requiring one carboxyl group and two benzyloxy functions appropriately placed for maximum $PGF_{2\alpha}$ antagonism in this series was a good assumption. A carbonyl rather than hydroxyl group at position C-1 of the indan is most beneficial for reversible antagonism. Reduction of the ketone to the alcohol is of synthetic interest and discussed in some detail.

Previous reports[2,3] from these laboratories describe our rationale for the design of keto-DIPA [5,6-bis(benzyloxy)-1-oxo-2-propyl-2-indanpropionic acid; 1] as a $PGF_{2\alpha}$ receptor antagonist. Noteworthy, the 15α-hydroxyl group of $PGF_{2\alpha}$, which is important for its agonist activity,[4] is replaced in 1 by a keto function having a juxtaposition to the carboxyl group similar to the juxtaposition of the 15α-hydroxyl function to the carboxyl group of $PGF_{2\alpha}$ but about one staggered ethylene moiety short of the distance between these groups in the proposed "active" hairpin conformation[5] of the prostaglandin. Studies in vitro[6] confirmed the ability of 1 to block $PGF_{2\alpha}$-induced contractions of the isolated uterus with an IC_{50} of 3.8×10^{-5} M. Investigations in vivo demonstrated the significant protective effects of 1 against the abortifacient actions of $PGF_{2\alpha}$ in mice,[3] as well as the absence of teratogenic activity.[7]

To explore further structural requirements for antagonistic activity in vitro, we prepared bis(benzyloxy) alcohol 2, a series of mono(benzyloxy) ketones (3–10) and alcohols (11–15), and diacid 22. Selected compounds were assessed for their pharmacological activity on the isolated rat uterus.

Chemistry. Keto acids 3, 4, and 6 were synthesized by using methods similar to those employed in the synthesis of keto acid 5.[2,6] Reduction of intermediate ester 9 with $NaBH_4$ in MeOH, however, did not afford the desired epimeric indanols 11 but rather two tricyclic compounds 17 (30%) and 18 (50%), which were separated by chromatography on silica gel using $CHCl_3$/*i*-PrOH (96:4) as eluting solvent. The indan C-1 proton resonance signals for 17 (δ 5.38) and 18 (δ 4.54) were particularly diagnostic. For lactones, the indan C-1 H resonance signal was consistently found to be downfield at approximately δ 5.4. Also, for 18, the additional two-proton resonance signal multiplets (X) bonded to the C α to oxygen appeared at δ 3.5–3.7. The [^{13}C]carbonyl resonance signal for 17 appeared at 172.4 ppm and the equivalent ^{13}C signal for 18 appeared at 63.5 ppm. Such a difference in chemical shift can be attributed to removal of electronegative oxygen. On the other hand, the C-1 indan ^{13}C resonance signals for 17 and 18 were identical (70.36 ppm). No OH stretching absorption was observed for 18 in the IR. Interestingly, when 17 was subjected to $NaBH_4$/MeOH reduction under conditions similar to those employed in the reduction of 9, a compound of identical R_f (0.34) [silica gel plates (5% hexane in $CHCl_3$)] with that of 18 was obtained as well as starting 17 (R_f 0.16). Further work would be necessary to elucidate the pathway of formation of 18 from 17. Thus, 17 may undergo solvolysis to ester 9 serving as intermediate to a diol precursor to 18. Reduction of other esters under similar conditions have previously been reported.[10]

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