# Synthesis and Biological Activity of Two Metabolites of 1-Methyl-5-(1-methylethyl)-2-nitro-1*H*-imidazole, an Antiprotozoal Agent

Bruno Cavalleri,* Giancarlo Volpe, Vittorio Arioli, and Giancarlo Lancini

*Research Laboratories, Gruppo Lepetit SpA, 20158 Milano, Italy.* *Received March 1, 1977*

5-(1-Hydroxy-1-methylethyl)-1-methyl-2-nitro-1*H*-imidazole (**6**) and 5-(1,2-dihydroxy-1-methylethyl)-1-methyl-2-nitro-1*H*-imidazole (**9**) are the principal metabolites found in urines of animals (mice, rats, and dogs) treated with 1-methyl-5-(1-methylethyl)-2-nitro-1*H*-imidazole (1), an effective antitrichomonas agent. These two metabolites have been synthesized. Compound **6** was found to be less toxic than the parent compound **1** and to possess essentially the same activity against *Trichomonas vaginalis* in experimental infections. Compound **9** showed a low degree of in vivo activity.

In a previous paper[1] we described the synthesis and the antitrichomonas activity of a series of 1,5-disubstituted 2-nitroimidazoles. Among these, 1-methyl-5-(1-methylethyl)-2-nitro-1*H*-imidazole (1) was the most active in oral treatment of *Trichomonas vaginalis* in mice. Recent studies[2] have shown that when this compound is given orally various metabolites may be isolated from the urines. The metabolites which are present in significant quantities are **6** (in mice, rats, and dogs) and **9** (in dogs) (Scheme I).

Metabolic oxidation of side chains has also been observed for 5-nitroimidazoles.[3-8] In addition, the appropriate isomer of compound **6**, 2-(1-hydroxy-1-methylethyl)-1-methyl-5-nitro-1*H*-imidazole, was found as a metabolite in tissues of animals whose feed was supplemented with 1-methyl-2-(1-methylethyl)-5-nitro-1*H*-imidazole.[9,10] On the other hand, both in 2-nitro- and 5-nitroimidazoles the *N*-methyl is not metabolically oxidized.[3,8-10] With regard to the nitro group, some authors did not see reduction products,[4,5] while others demonstrated condensation products derived from reduced intermediates.[11] Only in one case has an amino derivative been isolated.[12]

The metabolites of metronidazole[13] are essentially biologically inactive[6,14] and metabolites of nimorazole[15] have reduced activity when compared with the parent compound.[6] Comparisons[1] of the acute toxicities of 2-nitroimidazoles carrying alkyl side chains with those of the corresponding hydroxyalkyl derivatives confirmed the general rule that oxidation led to detoxification.

We have synthesized compounds **6** and **9** in order to confirm their structures and to evaluate their biological activity. Compound **6** was found to be less toxic than the parent compound (**1**) and had essentially the same activity against *T. vaginalis* in experimental infections. Therefore, it is under evaluation for potential therapeutic use.

**Chemistry.** The sequence of reactions involved in the synthesis of compounds **6** and **9** is indicated in Scheme I. The methylaminobutyronitrile (**3**) was prepared by treating the bisulfite addition product of $\alpha$-methoxyisobutyraldehyde (**2**) with $MeNH_2$, followed by KCN. Controlled catalytic reduction of **3** and hydrolysis of the intermediate imine gave the aldehyde **4** (not isolated), which was directly condensed in water solution with cyanamide under controlled conditions of pH.

As a consequence of partial cleavage of the *tert*-methoxyl group, the methoxyaminoimidazole **5** was obtained together with variable amounts of the corresponding hydroxy compound. By comparing $^1H$ NMR spectra ($Me_2SO$-$d_6$) of the crude mixture and of pure **5** (isolated by fractional crystallization), the relative amounts of the two compounds could be calculated. The $NCH_3$ protons of the hydroxyaminoimidazole resonate at lower field ($\Delta\delta$ 0.09 ppm) and the CH= and $NH_2$ protons resonate at higher field ($\Delta\delta$ 0.15 and 0.11 ppm, respectively). The IR spectrum (Nujol) of **5** was consistent with the tautomeric 2*H*-imidazole-2-imino-1,3-dihydro structure.

Replacement of the amino group with a nitro group was accomplished either on the mixture or on pure **5**. In both cases a further demethylation occurred and **6** was obtained. A small amount of the methoxy derivative **7** was isolated by chromatography. The latter could be hydrolyzed to **6** under mild conditions.

Treatment of **6** with dehydrating agents gave the methylethenyl derivative **8** which was converted into the diol **9** by oxidation with $KMnO_4$ in the presence of $MgSO_4$.[16] During this reaction a partial loss of the nitro group occurred and a dioxy compound (**10**) was isolated. The structure reported was consistent with the $^1H$ NMR spectrum ($Me_2SO$-$d_6$) and mass fragmentation.

Chemicophysical characteristics of **6** and **9** were in accordance with those of the corresponding metabolites isolated from urine of treated animals.

**Biological Results.** The in vitro and in vivo biological activities were studied using methods previously described (cf. ref 1 and 17). The $LD_{50}$ values were calculated according to the method of Litchfield and Wilcoxon.[18]

Compound **1**, chemically synthesized metabolites **6** and **9**, and the synthetic intermediates **7** and **8** were tested in vitro against *Staphylococcus aureus*, *Escherichia coli*, *Clostridium perfringens*, and *T. vaginalis* (Table I). Compound **6** showed moderate activity against both aerobes, while compound **1** was completely ineffective. The activity of **1** against *C. perfringens* and *T. vaginalis* was more or less maintained in the metabolites and intermediates.

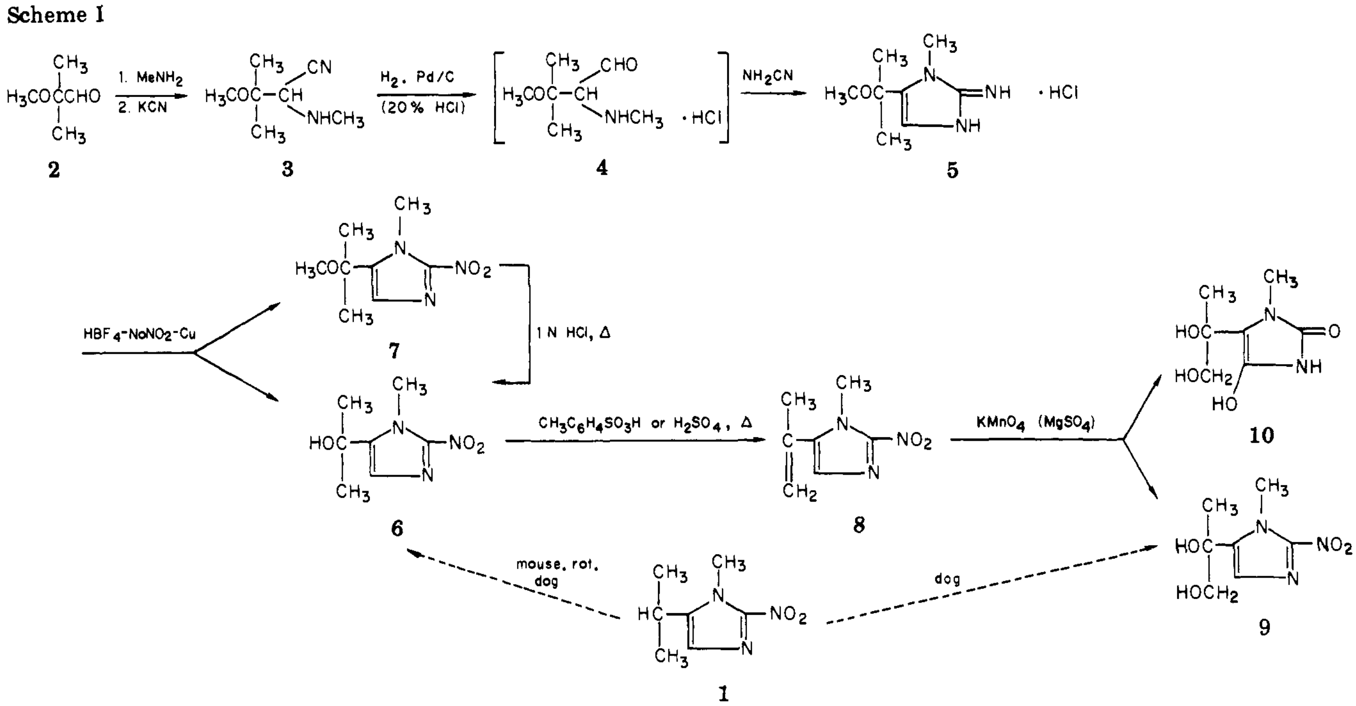
The $ED_{50}$'s of compounds **6** and **7** in *T. vaginalis* infection in mice were about the same as that of compound **1**, while compounds **8** and **9** were less active. As shown by the oral $LD_{50}$'s, **6** and **9** were significantly less toxic than **1**; compounds **7** and **8** had toxicities comparable with that of **1**. Thus, also in this case, metabolic oxidation led to a reduction of the acute toxicity.

Of particular interest was the fact that the therapeutic index of the major metabolite **6** was much higher than that of the parent compound **1**. This property suggests that compound **6** might have potential in human therapy.

## Experimental Section

Melting points (uncorrected) were determined in open capillary tubes or by differential scanning calorimetry (DSC). IR spectra were determined with a Perkin-Elmer Model 137 spectrophotometer as Nujol mulls. UV spectra were recorded in MeOH solution with a Unicam S.P. 800 spectrophotometer. $^1H$ NMR spectra were recorded at 60 MHz by a Varian A-60 spectrometer ($\delta$ relative to $Me_4Si$, 0.00 ppm). TLC were run on silica gel HF-$UV_{254}$ plates to a distance of 10.0 cm (the spots were detected by visual examination under UV light). Evaporation of solvents was done under reduced pressure using a rotary evaporator.

Scheme I




Analytical results for C, H, N, and, where applicable, for Cl were within ±0.4% of the theoretical values.

**3-Methoxy-3-methyl-2-methylaminobutyronitrile (3).** *α*-Methoxyisobutyraldehyde (**2**) (149.7 g, 1.46 mol) was added dropwise to a solution of 139 g (0.73 mol) of $Na_2S_2O_5$ in 150 mL of $H_2O$ at a temperature between -2 and 2 °C. After stirring for 1 h, 314 mL (3.25 mol) of 25% aqueous $MeNH_2$ was added. A slightly exothermic reaction occurred and the temperature was maintained at 5 °C by external cooling. After stirring for 1 h, 95 g (1.46 mol) of KCN was added in portions. The reaction mixture was stirred for 2 h at 10–15 °C, the solid was removed by filtration, and the aqueous solution was extracted with ether. The extracts were dried with $Na_2SO_4$ and evaporated. The residue was distilled collecting 118 g (56.6%) of **3**, which boils at 98–101 °C (20 mm). The hydrochloride was obtained by treating an ethanolic solution of **3** with ethereal HCl: mp 190–200 °C (from *i*-PrOH); IR 3100 (*ν* CH), 2800–1800 (*ν* $NH_2^+$), 1600 (*δ* $NH_2^+$), 1220 and 1175 ($CH_3CCH_3$), 1080 $cm^{-1}$ (*ν* COC); $^1H$ NMR ($Me_2SO$-$d_6$) *δ* 1.41 [s, 6 H, $(CH_3)_2C$], 2.70 (s, 3 H, $CH_3N$), 3.25 (s, 3 H, $CH_3O$), 5.0 (s, 1 H, CH), 8.8–10.2 (br, 2 H, mobile H). Anal. ($C_7H_{15}ClN_2O$) C, H, N, Cl.

**2-Imino-5-(1-methoxy-1-methylethyl)-1-methyl-1,3*H*-imidazole Hydrochloride (5).** A solution of 25.3 g (0.178 mol) of **3** in 135 mL of 20% HCl was hydrogenated on 3 g of 10% Pd/C at atmospheric pressure and room temperature with magnetic stirring. The theoretical amount of $H_2$ was adsorbed in 15 h. After filtering, the solution was brought to pH 4.6 with 10% NaOH and 11.5 g (0.27 mol) of $NH_2CN$ was added. The mixture was stirred at 60 °C for 2 h, maintaining the pH at 4.6 by addition of 10% HCl. Evaporation of the solution to dryness and washing with $Et_2O$ gave an oily residue which was extracted several times with anhydrous EtOH. After removal of the solvent, a residue was obtained which could either be utilized directly for the next step or crystallized twice from *i*-PrOH yielding 8.4 g (22.9%) of **5**. An analytical sample was recrystallized: mp 152 °C (DSC); IR 3500–2500 (*ν* $NH_2^+$), 1670 (*ν* C=N$^+$), 1600 and 1540 (*ν* C=N and C=C), 1220 and 1175 ($CH_3CCH_3$), 1060 $cm^{-1}$ (*ν* COC); $^1H$ NMR ($Me_2SO$-$d_6$) *δ* 1.43 [s, 6 H, $(CH_3)_2C$], 2.96 (s, 3 H, $CH_3O$), 3.50 (s, 3 H, $CH_3N$), 6.72 (s, 1 H, CH), 7.70 (br s, 2 H, $NH_2$). Anal. ($C_8H_{16}ClN_3O$) C, H, N, Cl. The **picrate** was obtained by adding to an aqueous solution of crude **5** an aqueous solution of picric acid, mp 204–205 °C (from EtOH).

**5-(1-Hydroxy-1-methylethyl)-1-methyl-2-nitro-1*H*-imidazole (6).** (a) A solution of 2.8 g (0.04 mol) of $NaNO_2$ in 11 mL of $H_2O$ was added dropwise at -20 °C in 15 min to a stirred solution of 7.75 g (0.037 mol) of **5** in 20 mL of $H_2O$ and 34 mL of 40% aqueous $HBF_4$. The stirring was continued for 15 min at -15 °C. The solution was kept at -15 °C and poured in portions into a well-stirred mixture of 8.2 g of Cu powder and 26.8 g (0.38 mol) of $NaNO_2$ in 395 mL of $H_2O$. After 30 min the insoluble matter was filtered off and the solution was brought to pH 2.5 with 10% HCl. $N_2$ was bubbled into the reaction mixture for 30 min and then was extracted with EtOAc. The organic extracts were washed with $NaHCO_3$ solution and with $H_2O$, dried ($Na_2SO_4$), and concentrated to a small volume. After standing 1.5 g of the title compound was obtained. The filtrate was evaporated and the residue was crystallized from benzene, obtaining an additional 0.48 g of **6**: mp 165 °C (DSC) (total yield 28%); IR 3270 (br, *ν* OH), 3140 and 3060 (*ν* CH=), 1535 and 1490 (*ν* C=C and C=C), 1525 ($\nu_{asym}$ $NO_2$), 1355 ($\nu_{sym}$ $NO_2$), 1350 (*δ* OH), 1150 (*ν* CO), 860 and 835 [*ν* $CN(O_2)$], 640 $cm^{-1}$ (*γ* OCN); UV $\lambda_{max}$ (log *ε*) 324 nm (3.98); $^1H$ NMR ($CDCl_3$) *δ* 1.58 [s, 6 H, $(CH_3)_2C$)], 4.20 (s, 3 H, $CH_3N$), 5.60 (s, 1 H, OH), 7.12 (s, 1 H, CH). Upon removal of the solvent from the mother liquor a mixture of **6** and **7** was obtained; these two compounds were separated by column chromatography as reported in the synthesis of **7**. (b) Compound **7** (0.2 g) was dissolved in 20 mL of 1 N aqueous HCl by heating at 80 °C for 10 min. The solvent was removed and the residue was dissolved in MeOH and reevaporated to give a solid which was crystallized from benzene: yield, 0.11 g of **6**. The product sublimes at 125 °C (0.5 mm).

**5-(1-Methoxy-1-methylethyl)-1-methyl-2-nitro-1*H*-imidazole (7).** Compound **3** (25.3 g, 0.178 mol) was treated as described for the preparation of **5**. After evaporation of the ethanolic extracts, a crude product was obtained which was characterized ($^1H$ NMR and TLC) as a mixture of **5** and of **2-imino-5-(1-hydroxy-1-methylethyl)-1-methyl-1,3*H*-imidazole hydrochloride**. This material was allowed to react as above. From the EtOAc extracts 14.5 g of an oily residue was obtained which by crystallization from benzene gave 5.9 g of **6**. The benzene mother liquor was evaporated and the residue was dissolved in $CHCl_3$ and chromatographed on 120 g of silica gel (0.06–0.2 mm). The $CHCl_3$ eluates were checked by TLC, developed with a 1:9 mixture of MeOH and $CHCl_3$ (**6** at $R_f$ 0.43; **7** at $R_f$ 0.70). An additional 1.7 g of **6** was recovered. Fractions containing compound **7** were collected and evaporated, and the residue was dissolved in benzene and rechromatographed through silica gel. The 5% EtOAc eluates gave 0.6 g of **7** (1.6%): mp 120–122 °C (from $Et_2O$–petroleum ether); IR 3050 (*ν* CH), 1530 and 1470 (*ν* C=N and C=C), 1520 ($\nu_{asym}$ $NO_2$), 1350 ($\nu_{sym}$ $NO_2$), 1220 and 1180 (*ν* $CH_3CCH_3$), 1065 (*ν* COC), 850, 840, and 820 $cm^{-1}$ [*ν* $CN(O_2)$]; UV $\lambda_{max}$ (log *ε*) 319 nm (3.94); $^1H$ NMR ($CDCl_3$) *δ* 1.66 [s, 6 H, $(CH_3)_2C$], 3.15 (s, 3 H, $CH_3O$), 4.21 (s, 3 H, $CH_3N$), 7.06 (s, 1 H, CH).

Table I



| Compd | R | Formula[a] | In vivo act. against selected organisms, MIC, µg/mL: *S. aureus* Tour | *C. perfringens* ISS 30453 | *E. coli* SKF 12140 | *T. vaginalis* Static | *T. vaginalis* Cidal | In vivo act.[c] against *T. vaginalis*, $ED_{50}$, mg/kg | Rel $ED_{50}$[d] | Acute toxicity[e] $LD_{50}$ (mice), mg/kg po |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $CH_3CHCH_3$ | $C_7H_{11}N_3O_2$[b] | >300 | 5 | >300 | 5 | 20 | 2.03 | 0.17 | 265 |
| 6 | $CH_3C(OH)CH_3$ | $C_7H_{11}N_3O_3$[b] | 100 | 25 | 100 | 6.2 | 25 | 2.5 | 0.21 | 698 (475 ip) |
| 7 | $CH_3C(OCH_3)CH_3$ | $C_8H_{13}N_3O_3$[b] | 100 | 100 | >100 | 6.2 | 50 | 2.18 | 0.19 | 350 (225 ip)[f] |
| 8 | $CH_2=CCH_3$ | $C_7H_9N_3O_2$[b] | >100 | 3.1 | >100 | 2 | 10 | 32.5 | 3.25 | 350[f] |
| 9 | $CH_3C(OH)CH_2OH$ | $C_7H_{11}N_3O_4$[b] | >100 | 5 | >100 | 10 | 20 | >80 | >8 | >1000 |
| | Metronidazole[g] | | >300 | 0.5 | >300 | 0.5 | 5 | 11.5 | 1 | 3800 |

[a] See the introduction to the Experimental Section. [b] Molecular weight confirmed by the $M^+$ peak in the mass spectrum. [c] Subcutaneous infection (mice), oral treatment. Details are given in ref 1. [d] The figures express the ratio between the $ED_{50}$ (compound) and the $ED_{50}$ (metronidazole) run in parallel. [e] Calculated according to the method of Litchfield and Wilcoxon.[18] [f] Approximate values. [g] See ref 13.

**1-Methyl-5-(1-methylethenyl)-2-nitro-1*H*-imidazole (8).** A mixture of 0.37 g of **6** and 0.34 g of *p*-toluenesulfonic acid in 70 mL of benzene was refluxed for 2 h. After cooling, the solution was washed with 10% $NaHCO_3$ and then with $H_2O$ and dried on $Na_2SO_4$. Removal of the solvent and crystallization (ether–petroleum ether) gave 0.24 g (71.8%) of **8**: mp 55–57 °C; TLC (developed with a 95:5 mixture of $CHCl_3$ and acetic acid) $R_f$ 0.83 (relative to **6**, $R_f$ 0.34); IR 3100, 3050 and 3020 ($\nu$ CH), 1635, 1540, and 1480 ($\nu$ C=N and C=C), 1520 ($\nu_{asym}$ $NO_2$), 1340 ($\nu_{sym}$ $NO_2$), 850 and 838 [$\nu$ $CN(O_2)$], 930 and 718 $cm^{-1}$ ($\gamma$ CH); $^1H$ NMR ($CDCl_3$) $\delta$ 2.13 [dd, 3 H, $J_{H_1-CH_3}$ = 1 Hz, $J_{H_2-CH_3}$ = 1.5 Hz, $CH_3C=C(CH_1H_2)$], 4.00 (s, 3 H, $CH_3N$), 5.29 and 5.56 (2 m, 2 H, $CH_2=$), 7.12 (s, 1 H, CH). Compound **8** was also prepared by heating a suspension of 5.4 g of **6** in 8 mL of concentrated $H_2SO_4$ for 45 min at 100 °C. After cooling, the reaction mixture was poured into ice and the pH was brought to 5–6 with concentrated $NH_4OH$. The solid was filtered, washed with water, dried in vacuo over $P_2O_5$, and recrystallized (yield 2.57 g, 52%). The product sublimes at 40 °C (1 mm).

**5-(1,2-Dihydroxy-1-methylethyl)-1-methyl-2-nitro-1*H*-imidazole (9).** A solution of 1.6 g (0.01 mol) of $KMnO_4$ and of 2.65 g (0.01 mol) of $MgSO_4 \cdot 7H_2O$ in 230 mL of $H_2O$ was added with stirring to a solution of 1.8 g (0.01 mol) of **8** in 180 mL of EtOH cooled at −10 °C. The reaction mixture was then stirred for 1 h at 0 °C and monitored by TLC. Since unreacted **7** was still present, 100 mL of ethanol was added and, after cooling to −10 °C, an additional 230 mL of $KMnO_4$–$MgSO_4$ solution was added. After filtering through Celite, the solvent was evaporated to dryness. An oily residue was obtained which was dissolved in a few milliliters of warm ethyl acetate, filtered from the insoluble matter, and concentrated to a small volume. A white crystalline product (**10**) was filtered off: 0.07 g; mp 206–209 °C (TLC developed with a 95:5 mixture of $CHCl_3$ and acetic acid, $R_f$ 0.74, visualized after spraying with $H_2SO_4$ and heating at 100 °C). The mother liquor was evaporated to dryness and the residue crystallized from ether, yielding 0.42 g (19.4%) of **9**: mp 96–98 °C; $R_f$ 0.78; IR 3450 and 3150 ($\nu$ OH), 1550 and 1495 ($\nu$ C=C and C=N), 1530 ($\nu_{asym}$ $NO_2$), 1350 ($\nu_{sym}$ $NO_2$), 1130 and 1070 ($\nu$ CO), 845 $cm^{-1}$ [$\nu$ $CN(O_2)$]; $^1H$ NMR ($Me_2SO$-$d_6$) $\delta$ 1.50 (s, 3 H, $CH_3C$), 3.52 and 3.61 (2 d, 2 H, $J_{gem}$ = 11.5 Hz, $J$ = 7 Hz, $CH_2$) 4.11 (s, 3 H, $CH_3N$), 4.90 (t, 1 H, $J_{CH_2OH}$ = 7 Hz, OH), 5.50 (s, 1 H, CH=).

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# Prostaglandin Prodrugs. 5.[1] Prostaglandin $E_2$ Ethylene Ketal

Moo J. Cho ,* Gordon L. Bundy, and John J. Biermacher

*The Upjohn Company, Kalamazoo, Michigan 49001.* *Received April 20, 1977*

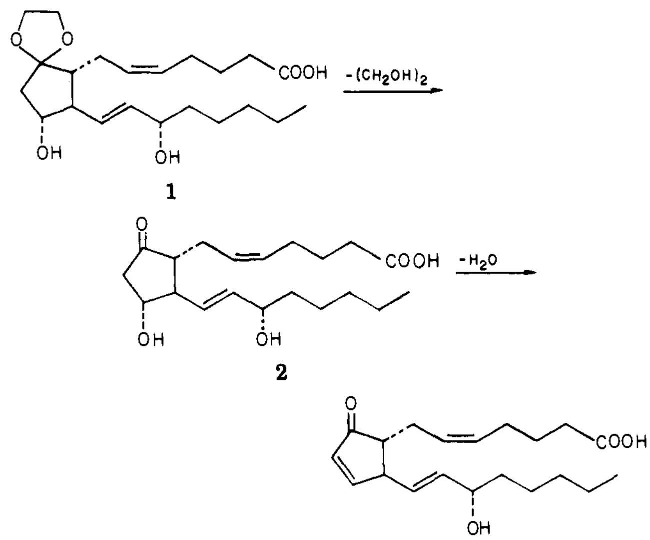
In order to improve the chemical stability of prostaglandin $E_2$ (**2**), prostaglandin $E_2$ ethylene ketal (**1**) was prepared by direct ketalization of **2** with ethylene glycol in benzene. To establish a quantitative assessment of **1** as a chemically stable and orally active prodrug of **2**, the hydrolysis of **1** to **2** and the subsequent dehydration of **2** to prostaglandin $A_2$ (**3**) were followed at 25 °C and six pH's ranging from 2.0 to 6.5 by means of a high-pressure liquid chromatographic procedure. Kinetic results clearly indicate that **1** should be quantitatively hydrolyzed back to the parent drug **2** under the stomach conditions without loss to **3**. At pH 2 and 25 °C, the half-lives of the hydrolysis of **1** to **2** and dehydration of **2** to **3** are in the order of 1 h and 14 days, respectively. The preliminary data on the biological response after oral administration of **1** appeared to indicate that **1** is bioequivalent to **2**.

The naturally occurring E and F prostaglandins have found a wide clinical application in human reproduction. For instance, prostaglandin $E_2$ (**2**, $PGE_2$) has been successfully used for labor induction and termination of pregnancy.[2] However, like most $\beta$-hydroxy ketones, the E series prostaglandins readily undergo dehydration to produce the A series prostaglandins[3] which have different spectra of biological activity.

$\beta$-Oxy cyclic ketones with various leaving groups of $pK_a$ ranging from 5 to 16 including $OH^-$ as in the case of **1** are known to undergo $\beta$-elimination through the E1cB mechanism,[4] and thus the dehydration of **2** is believed to occur in the same manner with either the formation of enolate at C-9 or the expulsion of $OH^-$ (or $H_2O$) from C-11 as the rate-determining step. Energetically, the fundamental driving force for the dehydration appears to be the reduction in free energy content derived from an extended conjugation present in prostaglandin $A_2$ (**3**, $PGA_2$). Therefore, any derivatives saturating the carbonyl group at C-9 of **2** should be prodrugs with excellent stability so long as they are converted to **2** with ease in the biological environment. For example, it was claimed that the reversible nucleophilic addition of bisulfite ion across the C-9 carbonyl group of **2** can improve the stability in aqueous solutions of neutral pH's.[5] Under acidic conditions the bisulfite adduct dissociates to rapidly release the parent prostaglandin.

In the present paper, we would like to report the synthesis of the C-9 ethylene ketal of **2**, which was found to be stable in the solid state, and its conversion to the parent compound **2** under acidic conditions, similar to the conditions encountered in the stomach. Implication is the possible use of **1** for an oral dosage form of **2**. To the present authors' knowledge, there are no enzymes in the blood stream which can effectively hydrolyze **1** to **2**, and, hence, the use of **1** in developing a parenteral solution of **2** appears to be of a remote possibility. Since both hydrolysis of **1** to **2** and dehydration of **2** to **3** are catalyzed by specific acid, the availability of **2** in the GI tract and ultimately in the blood stream will critically depend on the relative magnitude of both rate constants, $k_1$ for **1** to **2** hydrolysis and $k_2$ for **2** to **3** dehydration. For instance, if $k_2$ is much larger than $k_1$, the concentration of **2** will never be built up in the GI tract. The kinetics of **1** → **2** → **3** (Scheme I) was followed using a high-pressure liquid chromatographic (HPLC) procedure to establish a quantitative assessment of **1** as a chemically stable and orally active prostaglandin prodrug.

Scheme I




## Experimental Section

**Synthesis of $PGE_2$ Ethylene Ketal (1) (2 → 1).** A mixture of 1 g of $PGE_2$, 20 mL of freshly distilled ethylene glycol, and 100 mL of benzene was heated at reflux under nitrogen with vigorous stirring for 24 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted thoroughly with ethyl acetate. The combined organic layer was washed with water and brine, dried over sodium sulfate, and evaporated in vacuo. The crude product, 1.3 g, was taken up in 50 mL of methanol and treated under nitrogen with 20 mL of 3 N aqueous potassium hydroxide. [This conversion of unreacted $PGE_2$ to $PGB_2$ via $PGA_2$ (**3**) simplifies an otherwise very difficult chromatographic separation of $PGE_2$ and its ketal.] The mixture was allowed to stand for 2 h at room temperature and was then concentrated in vacuo to remove most of the methanol. The residue was diluted with ice and water, acidified with 35 mL of cold 2 N aqueous potassium bisulfate, and extracted thoroughly with ethyl acetate. The combined extracts were washed with water and brine, dried over sodium sulfate, and concentrated in vacuo. The 1.2-g residue was chromatographed on 90 g of Mallinckrodt CC-4 silica. Elution proceeded as follows (10-ml fractions): 500 mL of 50% ethyl acetate–Skellysolve B, fractions 1–50; 500 mL of 65% ethyl acetate–Skellysolve B, fractions 51–100; 500 mL of 80% ethyl