for the biological assays was provided by P. Betterman and E. Ware.

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Synthesis and Prostaglandin-like Activity of 2-(*trans*-3-Hydroxy-1-octenyl)-3-indoleheptanoic Acid

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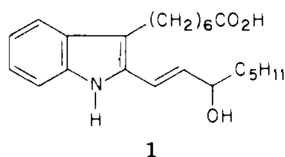
The synthesis of 2-(*trans*-3-hydroxy-1-octenyl)-3-indoleheptanoic acid (1) is described. The title compound appeared to show a weak prostaglandin-like activity in two different systems. It contracted rat stomach fundus strips and guinea-pig ileum preparations only at concentrations about 10^3- and 10^2-fold higher, respectively, than PGE_1. Moreover, it stimulated adenylate cyclase from rat liver plasma membrane, but the relative potency was 4–5 × 10^2-fold lower than the natural compound. The title compound showed also a certain degree of PGE_1 antagonism.

In recent years many synthetic analogues of prostaglandins, including derivatives of some heterocyclic systems, have been investigated.[1] We became particularly interested in an indole analogue which has the two common prostaglandin side chains attached to the C_2 and C_3 position (1). We chose this heterocycle since indole is a biological nucleus and 3-substituted indolealkanoic acids have shown interesting pharmacological actions.[2] Furthermore, analogue 1 has the indole ring system found in



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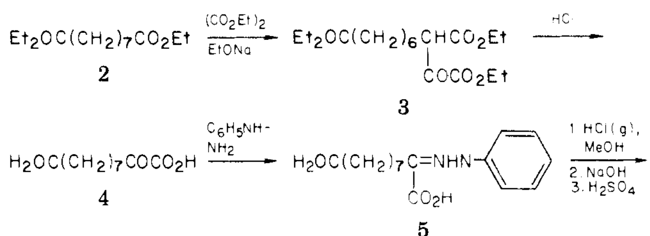
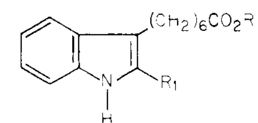
some nonsteroidal antiinflammatory drugs (e.g., indomethacin, which has been reported to inhibit the binding of PGE_1 to thymocytes[3] and to cell membrane fraction from bovine corpora lutea[4]) and so it might behave similarly with other prostaglandin receptors.

Conceptually one might consider the indole ring as being stereochemically equivalent to a 9,10-benzo analogue of the cyclopentane moiety in PGE_1, with the indole NH group mimicking the 11α-hydroxy. In addition, because the indole analogue 1 is devoid of the chiral centers which are present in the cyclopentane moiety of prostaglandins, it would allow easy preparation of substituted derivatives to follow any interesting biological activities found.

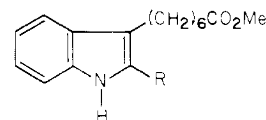
Chemistry. Condensation of diethyl azelate (**2**) with diethyl oxalate in the presence of sodium ethoxide in anhydrous diethyl ether afforded a 70% yield of the triester **3**, which, without purification, was hydrolyzed in 72% yield by means of diluted hydrochloric acid to the α-ketodicarboxylic acid **4**.

Scheme I





6, R = Me; R_1 = CO_2Me
7, R = H; R_1 = CO_2H



8, R = CO_2H
9, R = COCl
10, R = CH_2OH
11, R = CHO
12, R = CH=CHC(=O)C_5H_{11}
13, R = CH=CHCH(OH)C_5H_{11}

Fisher's indolization promoted by methanolic hydrochloric acid of the phenylhydrazone **5** obtained by action of phenylhydrazine on **4** (acetic acid–water, 3:2) produced the diester **6**, which, upon alkaline hydrolysis with aqueous–methanolic sodium hydroxide, gave a quantitative yield of the dicarboxylic acid **7** (see Scheme I). The latter was transformed into the half-ester **8** by treatment for 3

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Table I. Relative Potency of Compound 1 on Smooth Muscle Preparations

μg of 1	rat fundus (PGE_1 = 1)	guinea-pig ileum (PGE_1 = 1)
0.4	0.00075[a]	0.0040[a]
0.8	0.00075	0.0029
1.6	0.00056	0.0014
2.0	0.00055	0.0057

[a] Biological activity at every tested concentration represents the mean of five separate observations.

h at room temperature with methanol containing traces of toluene-*p*-sulfonic acid. The acid chloride **9** derived from **8**, by reaction with thionyl chloride in benzene solution, can be reduced with $NaBH_4$ in THF solution to the alcohol **10** which in turn was oxidized, with freshly prepared MnO_2 in ethereal solution, to the aldehyde **11**; the latter, however, can be obtained directly from **9** by reduction, at −40 °C in THF solution, with $LiAlH(O\text{-}t\text{-}Bu)_3$.

Wittig–Horner reaction with the sodium salt of dimethyl 2-oxoheptylphosphonate in anhydrous THF[5] gave the *trans*-enone **12** which was reduced to the alcohol **13** by treatment at 0 °C with $NaBH_4$ in methanol and finally transformed into the title compound **1** by mild alkaline hydrolysis.

Biological Activity. Since prostaglandins are known to induce a powerful contractile effect on smooth muscle, the biological activity of compound **1** on rat stomach fundus strip[6] and on guinea-pig ileum[7] was measured. The data on the intrinsic activity of analogue **1** are given in Table I.

As shown, compound **1** was less potent than PGE_1 in eliciting contractions of the two smooth muscles, approximately 5×10^2-fold in guinea-pig ileum and 1.5×10^3-fold in rat stomach fundus. Since it is not uncommon to see a weak agonist acting as a competitive inhibitor of a potent agonist at the receptor level, antagonist activity of the indole analogue **1** on rat stomach fundus was evaluated; the molar concentration of the derivative which inhibits by 50% contractions induced by a submaximally effective concentration of PGE_1 (11.3×10^{-9} mol/L) was approximately 11.8×10^{-6} mol/L.

Since many biological effects of prostaglandins are mediated by modifications of adenylate cyclase activity at the plasma membrane level,[8] we have investigated the effect of analogue **1** on cAMP levels. In broken-cell homogenates from liver, PGE_1 (2.8×10^{-5} mol/L) was found to induce a mean increase of cAMP levels by 300%. Equimolar doses of analogue **1** did not show any significant activity; only when tested at concentrations 4–500-fold higher than PGE_1, it elicited a stimulation of adenylate cyclase activity of the same order of magnitude with respect to the natural compound. Furthermore, preliminary experiments have shown that when PGE_1 (2.8×10^{-5} mol/L) is tested in the presence of a stimulatory concentration of analogue **1**, an inhibitory effect of about 40% on adenylate cyclase activity is seen as compared to PGE_1-induced stimulation. So the pattern of action of the indole analogue of PGE_1 seems to show dual characteristics. It has a low PGE_1-like activity but, at the same time, it shows a certain degree of PGE_1 antagonism.

Experimental Section

Melting points (Tottoli apparatus) are uncorrected. ^1H NMR spectra were recorded on a Hitachi Perkin-Elmer R24A spectrometer using Me_4Si as internal standard. IR spectra were determined on a Perkin-Elmer Model 257; where analyses are indicated by symbols, values were within ±0.4% of the calculated ones. Thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F_{254} plates.

α-Ketodecanedioic Acid (4). Diethyl oxalate (30 g, 205 mmol) and diethyl azelate (**2**) (55 g, 225 mmol) were added to a suspension of freshly prepared EtONa (14 g, 205 mmol, in 300 mL of diethyl ether). The mixture was stirred at room temperature for 48 h. Water (800 mL) was added and the organic layer was separated. The aqueous phase was acidified with HCl and the precipitated oil extracted with diethyl ether. After the usual workup, the crude residue (55 g) was refluxed with 750 mL of 6 N HCl for 12 h. The solution was decolorized with charcoal and evaporated in vacuo to dryness. The solid was crystallized from diethyl ether–petroleum ether (3:1) to afford 24 g of **4**, mp 90–91 °C (54%). Anal. ($C_{10}H_{16}O_5$) C, H.

2-Carboxy-3-indoleheptanoic Acid (7). A mixture of phenylhydrazine (2.16 g, 20 mmol) and α-ketodecanedioic acid (**4**, 4 g, 18.5 mmol) in 30 mL of acetic acid–water (6:4) was heated at 40–45 °C for 45 min. Water was added and the precipitated solid was filtered and crystallized from methanol–water (1:1) to afford 4.7 g of **5**, mp 130–131 °C (83%). The latter, dissolved in MeOH (60 mL), was saturated with dry HCl and the mixture left at room temperature for 48 h. Water was added and the precipitated oil extracted with benzene. After drying, the solvent was removed in vacuo and the residue chromatographed on a silica gel column. Elution with Et_2O–hexane (4:6) gave 2 g of **6**: mp 53–54 °C (41%); IR (Nujol) 3370, 1725, 1680 cm^{-1}; NMR ($CDCl_3$) 3.67 (s, 3 H, $-COOCH_3$), 3.90 (s, 3 H, $-COOCH_3$), 9.15 ppm (s, 1 H, NH).

The diester, dissolved in MeOH (15 mL), was refluxed for 2 h with 0.5 g of NaOH in 10 mL of water. MeOH was removed in vacuo and the aqueous layer acidified with 6 N HCl. The precipitated solid was filtered to afford 1.8 g of **7**, mp 141–142 °C, after crystallization from benzene–ligroine (1:1): IR (Nujol) 3340, 1690 cm^{-1}. Anal. ($C_{16}H_{19}NO_4$) C, N, H.

Methyl 2-Carboxy-3-indoleheptanoate (8) and the Corresponding Acid Chloride 9. A mixture of 0.18 g of **7** (0.62 mmol) in MeOH (10 mL) containing 0.01 g of *p*-TosOH (0.06 mmol) was stirred at room temperature for 3 h. After removal of MeOH in vacuo, the residue, on crystallization from benzene–ligroine (2:1), gave 0.12 g of **8**: mp 119–120 °C (63.6%); IR (Nujol) 3380, 1725, 1650 cm^{-1}; NMR ($CDCl_3$) 3.65 (s, 3 H, $-COOCH_3$), 9.05 (br s, 1 H, NH), 10.6 ppm (s, 1 H, $-COOH$). Anal. ($C_{17}H_{21}NO_4$) C, H, N.

The half-ester **8** (0.6 g, 1.98 mmol) was suspended in benzene (10 mL) and heated at 50 °C for 2 h with thionyl chloride (0.5 mL). Evaporation in vacuo gave a solid residue which was triturated with petroleum ether and filtered to yield 0.5 g of **9**: mp 49–50 °C (79%); IR (Nujol) 3340, 1800, 1700 cm^{-1}. Anal. ($C_{17}H_{20}NO_3Cl$) C, H, N, Cl.

Methyl 2-Formyl-3-indoleheptanoate (11). (A) A solution of **9** (1.3 g, 4 mmol) in THF (15 mL) was added dropwise at 0 °C to a well-stirred solution of $NaBH_4$ (1 g, 26 mmol) in water (8 mL). The mixture was left at room temperature for 2 h and then extracted with Et_2O. The organic extracts, after washing with saturated brine, were dried ($MgSO_4$) and evaporated in vacuo to give 0.9 g of **10** (76.9%): IR (Nujol) 3500–3300, 1730 cm^{-1}; NMR ($CDCl_3$) 3.67 (s, 3 H, $-COOCH_3$), 4.75 (s, 2 H, $-CH_2OH$), 9.00 ppm (s, 1 H, NH).

A solution of **10** (0.3 g, 1.04 mmol) in Et_2O (50 mL) was stirred at room temperature in the presence of MnO_2 (3.5 g) for 2 h and then filtered. The filtrate was evaporated in vacuo to get the aldehydo ester **11** (0.25 g) as a solid: mp 60–61 °C (84%); IR (Nujol) 3300, 1730, 1645 cm^{-1}; NMR ($CDCl_3$) 3.65 (s, 3 H, $-COOCH_3$), 9.4 (s, 1 H, NH), 10.1 ppm (s, 1 H, $-CHO$).

(B) A solution of **9** (0.5 g, 1.54 mmol) in THF (5 mL) was added at −40 °C to a suspension of $LiAlH(O\text{-}t\text{-}Bu)_3$ (0.41 g, 1.62 mmol) in THF (10 mL). After 1 h, the mixture was gradually allowed to warm at room temperature for 1 h. Water (3 mL) and then diluted HCl (10 mL) were added and the mixture was extracted with Et_2O. After the usual workup, **11** was obtained as a solid, mp 60–61 °C (0.29 g, 64%). Anal. ($C_{17}H_{21}NO_3$) C, H, N.

Methyl 2-(*trans*-3-Hydroxy-1-octenyl)-3-indoleheptanoate (12). Dimethyl 2-oxoheptylphosphonate (0.28 g, 1.26 mmol) was dissolved in dry THF (5 mL) and treated with NaH (50% oil) (0.064 g, 1.32 mmol) at 0 °C for 10 min and then for 1 h at room temperature. A solution of **11** (0.35 g, 1.2 mmol) in dry THF (5 mL) was then added dropwise with stirring at 0 °C and allowed to warm at room temperature. After 3 h the mixture was poured

into ice–water and extracted with Et_2O. The Et_2O solution was dried ($MgSO_4$) and evaporated to afford **12** (0.44 g) as a solid: mp 69–70 °C (95%); IR ($CHCl_3$) 3470, 1730, 1640, 1590 cm^{-1}; NMR ($CDCl_3$) 0.85 (t, 3 H, J = 6 Hz, CH_3), 3.65 (s, 3 H, $-COOCH_3$), 6.55 (d, 1 H, J = 16 Hz, –CH=C*H*CO), 7.70 (d, 1 H, J = 16 Hz, –C*H*=CHCO), 8.82 ppm (s, 1 H, NH). Anal. ($C_{24}H_{33}NO_3$) C, H, N.

Methyl 2-(*trans*-3-Hydroxy-1-octenyl)-3-indoleheptanoate (13). To a solution of **12** (0.3 g, 0.78 mmol) in MeOH (20 mL) solid $NaBH_4$ (0.1 g, 2.6 mmol) was added portionwise at 0 °C. After 1 h the mixture was diluted with water and extracted with Et_2O. Usual workup gave **13** (0.25 g) as an oil after chromatographic purification on silica gel (eluent: petroleum ether–Et_2O, 3:1) (83%): IR ($CHCl_3$) 3480, 3400–3350, 1730, 1610 cm^{-1}; NMR ($CDCl_3$) 0.85 (t, 3 H, J = 6 Hz, CH_3), 3.65 (s, 3 H, $-COOCH_3$), 4.1–4.5 (br, 1 H, C*H*OH), 5.95 (dd, 1 H, J = 16 Hz, J = 7 Hz, –CH=C*H*CHOH), 6.65 (d, 1 H, J = 16 Hz, –C*H*=CHCHOH), 8.60 ppm (br, 1 H, NH).

2-(*trans*-3-Hydroxy-1-octenyl)-3-indoleheptanoic Acid (1). The hydroxy ester **13** (0.2 g, 0.52 mmol) in MeOH (16 mL) was refluxed with 16 mL of an aqueous 10% solution of K_2CO_3 for 2 h. The solution was concentrated in vacuo, diluted with water, and acidified with 2 N HCl. The precipitated solid was collected by filtration and crystallized from Et_2O to yield 0.14 g of **1**: mp 106–107 °C (73.7%); IR ($CHCl_3$) 3480, 1710, 1610 cm^{-1}; NMR ($CDCl_3$) 0.87 (t, 3 H, J = 6 Hz, CH_3), 4.25 (m, 1 H, C*H*OH), 6.02 (dd, 1 H, J = 16 Hz, J = 7 Hz, –CH=C*H*CHOH), 6.60 (d, 1 H, J = 16 Hz, –C*H*=CHCHOH), 8.97 ppm (br, 1 H, NH). Anal. ($C_{23}H_{33}NO_3$) C, H, N.

Bioassay. The rat stomach fundus strip was suspended in an organ bath (4 mL) at 36 °C in Krebs solution gassed with 95% O_2 and 5% CO_2 containing 3×10^{-9} mol/L of cyproheptadine as antagonist of 5-hydroxytryptamine and histamine and 2.8×10^{-6} mol/L of indomethacin as endogenous PGs synthesis inhibitor. Drug or PGE_1 standards were added to the bathing solution as soon as the preparation reached a constant tone. Contractions were recorded on a smoked kymograph paper using an auxotonic lever with a 1 × 20 magnification. The baseline load was 1 g, maximal 3 g. The dose cycle was 10 min, with a contact time of 90 s.

The terminal ileum was set up in a 3-mL bath in oxygenated Tyrode solution at 30 °C, containing 3×10^{-9} mol/L of cyproheptadine. The assay was done at 5-min intervals, with a contact time of 30 s. Contractions were recorded with an isotonic lever with a 1 × 20 magnification, writing on a smoked drum. Tissues were loaded at 0.4–0.7 g.

The amount of PGE_1-like activity of analogue **1** was obtained by bracketing its response between those of two known doses of PGE_1 standards. Compound **1** and PGE_1 standards were dissolved in ethanol, diluted in Krebs or Tyrode solution, and added to the organ baths in a volume of 0.1 mL to give the following final concentrations: compound **1**, 2.70–13.5 × 10^{-7} mol/L, and PGE_1 standards, 0.70–14.0 × 10^{-9} mol/L, in the rat fundus bath; and compound **1**, 3.6–17.9 × 10^{-7} mol/L, and PGE_1 standards, 0.94–18.8 × 10^{-9} mol/L, in the guinea-pig ileum bath.

The log dose–response curve for PGE_1 was linear for both the smooth muscles in the tested concentration range. According to Tolman et al.,[9] antagonist activity of compound **1** was determined by comparing the magnitude of rat fundus strip contractions induced by 11.3×10^{-9} mol/L of PGE_1, in the presence and in the absence of different concentrations of the indole analogue (2.15–21.5 × 10^{-6} mol/L). The approximate IC_{50} was obtained from the concentration–response curve of analogue **1** as an inhibitor of PGE_1-induced rat fundus strip contractions in four separate preparations.

Rat Liver Homogenate Prostaglandin Assay. Adenylate cyclase activity of rat liver homogenates was assayed by an indirect method[10] measuring the cAMP produced by transformation of ATP under catalysis of the enzyme.

Rat liver homogenates were incubated for 10 min at 37 °C in a medium containing (mol/L) ATP, 4×10^{-3}; $MgSO_4 \cdot 7H_2O$, 15×10^{-3}; Tris–HCl, 0.1 (pH 8.0); GTP, 5×10^{-4}; EGTA, 1×10^{-4}; theophylline, 5×10^{-3}; PGE_1, 2.8×10^{-5}; compound **1**, 6.7–13.4 × 10^{-3}; and NaCl, 0.9% for blanks. The final volume was 0.4 mL.

The reaction was terminated by immersing the tubes in boiling water for 2 min. Tubes were frozen at –20 °C. After thawing, samples were centrifuged at 1200*g* for 10 min and the supernatants assayed for cAMP according to the method of Brown et al.[11] The experiment was replicated four times. Proteins were measured according to the method of Lowry et al.[12]

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Synthesis and Xanthine Oxidase Inhibitory Analysis of 1*H*-Pyrrolo[3,2-*c*]pyridine-4,6(5*H*,7*H*)-dione (3,7-Dideazaxanthine) and Two of Its Derivatives

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The synthesis of 1*H*-pyrrolo[3,2-*c*]pyridine-4,6(5*H*,7*H*)-dione (3,7-dideazaxanthine) (**1**), 5-methyl-1*H*-pyrrolo-[3,2-*c*]pyridine-4,6(5*H*,7*H*)-dione (1-methyl-3,7-dideazaxanthine) (**2**), and 1,7-dihydropyrano[4,3-*b*]pyrrole-4,6-dione (1-oxa-1,3,7-trideazaxanthine) (**3**) has been accomplished from 3-alkoxycarbonylpyrrole-2-acetates (**4**, **11**, and **12** for **1** and **2**) and from 3-carboxypyrrole-2-acetic acid (**6** for **3**). Compounds **1** and **2** have been found to be weak inhibitors of the noncompetitive type for xanthine oxidase while **3** showed no inhibitory properties toward this enzyme.

Investigations into deazapurines and their nucleosides have produced much revealing information about the biological roles of the ring nitrogen atoms in the metabolic functions of purine systems while also providing several derivatives of potential biological significance.[4–6] In our effort to organize and rationalize these diverse results for

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