of the aliphatic amides noted above was obtained when modified E_s values were used along with a parameter explicity allowing for hyperconjugative effects[8] rather than using the single parameter, E_s.

The rate constants in Table II are overall rate constants, *i.e.*, a composite for steps 3 and 4. Buglass, *et al.*,[5] have calculated rate constants for step 4 for the acidic hydrolysis of a series of para-substituted benzohydroxamic acids and report a positive Hammett ρ value for correlation of those rate constants, a result consistent with the bimolecular mechanism. An examination of the data in their[5] Table 6 indicates at best (with the *p*-hydroxy compound excluded) only a fair correlation between the observed overall rate constants and Hammett σ constants with a negative value for ρ. This result is consistent with our negative value for ρ^* for the overall rate constants for the aliphatic compounds.

Since $\rho^* < 0$ in eq 5, electron-donating groups accelerate the rate compared to that of the reference compound, acetohydroxamic acid. This is consistent with the greater electronegativity of hydroxyl compared to hydrogen in changing from amides to hydroxamic acids, provided that the polar effect on the protonation of hydroxamic acids is greater than the polar effect for the nucleophilic attack by water on the protonated intermediate in the bimolecular mechanism. The positive value for δ means that steric effects are rate decelerating compared to acetohydroxamic acid as would be anticipated.

Experimental Section

Aceto-, isobutyro-, and pivalohydroxamic acids have been described previously.[10] Propionohydroxamic acid was prepared by adaptation of the method used for preparation of isobutyrohydroxamic acid, purified by means of the copper salt, and crystallized from ethyl acetate, mp 93.2–95.0° (lit.[11] mp 92.5–93°). Phenylacetohydroxamic acid, mp 142.7–144.0° dec (lit.[12] mp 143–144° dec), was prepared by adaptation of the method used for benzohydroxamic acid.[4]

The 0.494 *M* *p*-toluenesulfonic acid solution (Table I) was prepared by addition of the acid to distilled water and titrated with standardized base. The 0.247 and 0.124 *M* solutions were prepared from the above solution by appropriate dilutions and with potassium chloride added to maintain the ionic strength at 0.494 *M*. The 0.249 *M* *p*-toluenesulfonic acid (Table II) was prepared by addition of the acid to double distilled water and titrated as above.

Kinetic measurements were made by use of the spectrophotometric method reported previously[4] using either a photoelectric colorimeter[4] (Table I) or a Beckman DU spectrophotometer (Table II) set at 520 nm. Pseudo-first-order rate constants were obtained from the slope of the appropriate graph.[4] The rate constants reported in column two in Table I are the average of five, two, and six runs, respectively, from highest to lowest catalytic acid concentration. The rate constants in Table II are averages of duplicate or triplicate measurements. Average deviation from the mean is less than 1.7%. Temperature control was ±0.05°. Initial concentration of hydroxamic acids in the kinetic runs was 0.012 *M*.

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Microbiological Reduction and Resolution of Prostaglandins. Synthesis of Natural PGF$_{2}\alpha$ and *ent*-PGF$_{2}\beta$ Methyl Esters

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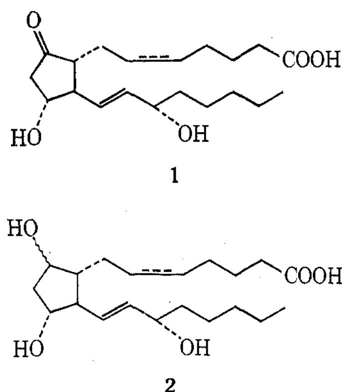
The total synthesis of racemic prostaglandins E_1 (**1**, 5,6-saturated) and E_2 (**1**, 5,6-cis double bond) and their



methyl esters *via* bicyclo[3.1.0]hexane intermediates has previously been reported from these laboratories.[1] Chemical reduction of the 9-keto group of these compounds using sodium borohydride led to racemic PGF$_{1}\alpha$ (**2**, 9α,5,6-saturated), PGF$_{1}\beta$ (**2**, 9β,5,6-saturated), and PGF$_{2}\alpha$ (**2**, 9α,5,6-cis double bond), PGF$_{2}\beta$ (**2**, 9β,-5,6-cis double bond), respectively. Natural PGF$_{1}\alpha$ and PGF$_{2}\alpha$ have the 9*S* configuration while nat-PGF$_{1}\beta$ and PGF$_{2}\beta$ are 9*R*. Fermenting yeasts are known to reduce ketones to optically active secondary alcohols of the *S* configuration, the extent of stereoselectivity varying somewhat with the steric environment of the keto group.[2] Enzymatic reductions of some steroid ketones show high stereoselectivity.[3] It was thus of interest to us to determine the effect of enzymes of fermenting yeasts and other microorganisms on prostaglandins E_1 and E_2. Stereoselective microbiological reduction of a racemic prostaglandin 15-ketone **3** to **4** has recently been reported.[4]

Actively fermenting baker's yeast was found to reduce *nat*-PGE$_1$ and *nat*-PGE$_2$ slowly to PGF$_{1}\alpha$ and PGF$_{2}\alpha$, respectively. No appreciable amounts of the 9β epimers could be seen by thin layer chromatography of extracts, thus demonstrating the stereoselective

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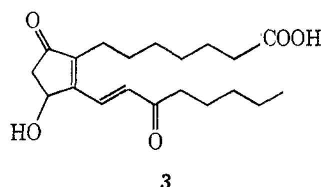

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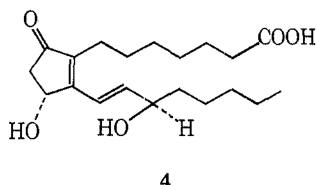

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nature of the reduction to 9-alcohols of the *S* configuration. The methyl esters of *nat*-PGE_1 and PGE_2 were slowly hydrolyzed by the same fermentation mixture prior to reduction of the 9-ketone, also producing $PGF_{1}\alpha$ and $PGF_{2}\alpha$.

When *rac*-PGE_1 methyl ester and *rac*-PGE_2 methyl esters were subjected to the same conditions, tlc spots corresponding in mobility and color reactions to both isomeric 9-alcohols (*i.e.*, $PGF_{1}\alpha$ and $PGF_{1}\beta$ from PGE_1 methyl ester and $PGF_{2}\alpha$, $PGF_{2}\beta$ from PGE_2 methyl ester) were observed. These pairs of products were produced in about equal amounts, suggesting that yeast reduced both enantiomers of the racemates, producing *nat*-$PGF_{1}\alpha$, *ent*-$PGF_{1}\beta$, and *nat*-$PGF_{2}\alpha$, *ent*-$PGF_{2}\beta$, respectively. This was confirmed by the isolation of the products from the reduction of *rac*-PGE_2 methyl ester by silica gel chromatography of their methyl esters. The $PGF_{2}\alpha$ methyl ester obtained gave a positive plain ORD curve of the same shape as that of *nat*-$PGF_{2}\alpha$ methyl ester and of nearly the same amplitude. The $PGF_{2}\beta$ methyl ester was crystalline, mp 85–87° (*vs.* 90–91° for *nat*-$PGF_{2}\beta$ methyl ester), but had an ORD curve which was the mirror image of that exhibited by *nat*-$PGF_{2}\beta$ methyl ester. The amplitudes of the ORD curves indicated about 85% optical purity, assuming that the only impurity is the optical antipode.

Thus, the stereoselective microbiological reduction, hydrolysis, and resolution of racemic PGE_1 and PGE_2 methyl esters has been demonstrated. The isolated yield of *nat*-$PGF_{2}\alpha$ was only about 10%, however, and the yield was not improved by the use of a special enriched growth medium.[2b] Screening of other microorganisms and conditions also failed to improve the yield, although Torulopsis yeast also reduced and hydrolyzed *rac*-PGE_2 methyl ester. These yeast reductions were quite slow, with starting PGE_2 still present after 46 hr at 25°, and undesired side reactions were evident, such as dehydration to PGA_2 and reduction of the terminal carboxyl group.

Experimental Section

Yeast Reduction of *rac*-PGE_2 Methyl Ester.—A total of 500 mg of *rac*-PGE_2 methyl ester was reduced by yeast in four identical batches, each one as follows. A mixture of 200 ml of boiled water, 25 g of sugar, and 1 cake (17.5 g) of baker's yeast was allowed to incubate at 25° for 0.75 hr, when CO_2 evolution through a water bubbler was rapid. Then a solution of 125 mg of the substrate in 5 ml of ethanol was added. The mixture was stirred and samples (10 ml) were withdrawn at intervals. These were acidified with 1 ml of 3 *N* HCl, shaken with ethyl acetate, and filtered, and the ethyl acetate layer was evaporated to leave a residue which was assayed by thin layer chromatography (silica gel plates, developed by AIX system[5] and visualized by spraying and heating with a vanillin–phosphoric acid spray[6]). After 20 hr, most of the starting PGE_2 methyl ester had been hydrolyzed to PGE_2 and minor spots corresponding in tlc mobility and color reactions to PGA_2, $PGF_{2}\alpha$, and $PGF_{2}\beta$ were seen, the latter two of about equal intensity. After 29 hr, 25 g more sugar was added and at 46 hr, while the $PGF_{2}\alpha$ and $PGF_{2}\beta$ spots had increased in intensity, there was still much PGE_2 left as judged by tlc. The mixture was worked up in the same way as for the aliquots above and the crude products were chromatographed on 50 g of acid-washed silica gel. Elution with 40–100% ethyl acetate in Skellysolve B gave 304 mg of *rac*-PGE_2 and 106 mg of material consisting of a mixture of $PGF_{2}\alpha$ and $PGF_{2}\beta$. This latter mixture was treated with excess ethereal diazomethane and rechromatographed on 10 g of silica gel. The column was eluted with ethyl acetate and 1 and 2% methanol in ethyl acetate. There was obtained 25 mg of noncrystalline material, homogeneous by tlc, spectrally identical with $PGF_{2}\alpha$ methyl ester (ir and nmr) and showing a plain positive ORD curve in EtOH, $[\alpha]_{589}$ +18.3°, $[\alpha]_{220}$ +440° (for *nat*-$PGF_{2}\alpha$, $[\alpha]_{589}$ +25°, $[\alpha]_{220}$ +534°, EtOH).

The more polar material (28 mg) was crystalline, and melted at 85–87° after two recrystallizations from ethyl acetate–Skellysolve B. This was spectrally (ir, nmr) identical with $PGF_{2}\beta$ methyl ester but had an ORD curve which is positive at long wavelengths, becoming negative below 320 nm, $[\alpha]_{589}$ +5.6°, $[\alpha]_{220}$ −995°, and is the mirror image of that of *nat*-$PGF_{2}\beta$ methyl ester, $[\alpha]_{589}$ −5.2°, $[\alpha]_{220}$ +1400°.

Yeast Reduction of *rac*-PGE_1 Methyl Ester.—In the same manner as the preceding experiment, 120 mg of *rac*-PGE_1 methyl ester was reduced. After 3 hr, partial hydrolysis to *rac*-PGE_1 was seen by tlc of an aliquot, and at 22 hr, additional spots corresponding in mobility and color reactions to PGA_1, $PGF_{1}\alpha$, and $PGF_{1}\beta$ were seen. At 29 hr, 25 g more sugar was added and the mixture was worked up as before at 50 hr. The crude residue after evaporation of extracts was chromatographed on 50 g of Silicar CC_4 (Mallinckrodt) silica gel, eluting with solvent mixtures ranging from 50% ethyl acetate–Skellysolve B to 5% methanol–ethyl acetate. Fractions 13–18 contained 10 mg of material which was partially crystalline, resembled $PGF_{1}\alpha$ on thin layer plates, and showed a positive rotation as does $PGF_{1}\alpha$, but was not further purified. Fractions 20–23 contained an equal quantity of material with thin layer behavior like that of $PGF_{1}\beta$, also showing a small positive rotation (*nat*-$PGF_{1}\beta$ has $[\alpha]^{25}D$ −20°, EtOH).[7]

Yeast Reductions of *nat*-PGE_1 and *nat*-PGE_2.[8]—In the same manner as the preceding experiment, 250 mg of *nat*-PGE_1 was incubated with fermenting yeast. After 30 hr, tlc spots corresponding in mobility and color reactions to PGA_1, PGE_1, and $PGF_{1}\alpha$ were seen, but no $PGF_{1}\beta$ was evident. Work-up as above, treatment with diazomethane, and chromatography on 25 g of silica gel gave 183 mg of *nat*-PGE_1 methyl ester followed by 10 mg of *nat*-$PGF_{1}\alpha$ methyl ester, identical in tlc color and mobility and ir spectra with authentic materials. Further elution of the column failed to elute any material resembling $PGF_{1}\beta$ methyl ester on tlc plates.

On a smaller scale, reduction of 10 mg of *nat*-PGE_2 gave material showing tlc spots corresponding in mobility and color with starting PGE_2 and $PGF_{2}\alpha$. An identical reduction of 10 mg of *rac*-PGE_2 methyl ester showed, in addition, a spot on tlc like $PGF_{2}\beta$ of approximately the same intensity as the $PGF_{2}\alpha$ spot. Treatment of the extract with ethereal diazomethane converted these to materials having the same mobility as that of $PGF_{2}\alpha$ and $PGF_{2}\beta$ methyl esters.

Registry No.—(±)-PGE_2 (Me ester), 31660-08-9; *nat*-$PGF_{2}\alpha$, 551-11-1; mirror image of *nat*-$PGF_{2}\beta$ (Me ester), 37107-45-2; (±)-PGE_1 (Me ester), 20993-69-5; *nat*-$PGF_{1}\alpha$, 745-62-0; *nat*-$PGF_{1}\beta$, 10164-73-5; *nat*-PGE_1, 745-65-3; *nat*-PGE_2, 363-24-6.

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