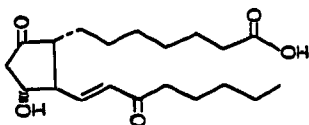


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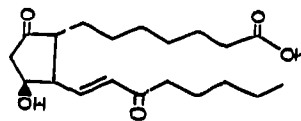
## Note

### Separation of prostaglandin diastereomers by countercurrent distribution

In a recent publication<sup>1</sup> on the total synthesis of  $\text{PGF}_{1\alpha}$ , 15-keto- $\text{PGE}_1$  and 11-*epi*-15-keto- $\text{PGE}_1$  were separated by partition chromatography using Mallinckrodt Silic AR CC-4 as the solid support. This separation was not complete, requiring rechromatography of the mixture. Counter-current distribution has been used to provide reproducibly a complete separation of the diastereomers in a single run.



15-keto- $\text{PGE}_1$



11-*epi*-15-keto- $\text{PGE}_1$

#### Apparatus and materials

**Counter-current distribution apparatus.** The distributions were performed by a robot-operated 240-cell counter-current distribution (H. O. Post Scientific Instrument Co.). The cell capacity was 3 ml of lower phase and a maximum of 5 ml of upper phase.

**Solvents.** Prior to use, commercially available benzene and methanol were distilled. The water was deionized and distilled.

#### Counter-current distribution procedure

In preparation for a run, the solvents were mixed and allowed to equilibrate. Each cell was filled with 3 ml of lower phase, except the cells that contained the sample.

The diastereomeric mixture was dissolved in 3 ml of upper phase and 3 ml of lower phase. The sample was placed in cell zero. The automatic filling device was set to deliver 3 ml of upper and 0.2 ml of lower phase. The operating conditions for counter-current distribution were decantation of upper phase, transfer of upper phase, 20 equilibration strokes, a 2-min settling period and repetition of the cycle until the desired number of transfers was achieved.

Initially, 240 transfers were performed to evaluate the solvent system. Thin layer chromatography (TLC) was used to monitor the results at the end of 240 transfers. If more transfers were necessary, as was generally the case, a recycling tube was connected to the counter-current distribution apparatus and the additional transfers were performed.

#### TLC procedure

The plates were made of Woelm Silica Gel F with 1.5% Vee Gum binder. The solvent system was benzene-ethyl acetate-acetic acid (50:50:2). The reagents for making the spots visible were 50%  $\text{H}_2\text{SO}_4$ - $\text{H}_2\text{O}$  and 10% (w/v) phosphomolybdic acid-ethanol.

*Results and discussion*

The partition ratios<sup>2</sup> (see Table I) were calculated for the 15-keto-PGE<sub>1</sub> and 11-*epi*-15-keto-PGE<sub>1</sub> by using the equation

$$K = N/(n - N)$$

where  $N$  is the peak tube and  $n$  is the number of transfers.

The separation factor<sup>3</sup> ( $\beta$ ) is used to predict the relative ease with which a separation can be achieved. The larger  $\beta$  is, the easier the separation. When  $\beta$  is unity, there is no separation. The separation factor was calculated for each solvent system by using the equation

$$\beta = K_1/K_2$$

where  $K_1 = K$  for 11-*epi*-15-keto-PGE<sub>1</sub> and  $K_2 = K$  for 15-keto-PGE<sub>1</sub>

TABLE I  
PARTITION RATIOS OF 15-KETO-PGE<sub>1</sub> AND 11-*epi*-15-KETO-PGE<sub>1</sub>

System	Solvent ratios			$K_1$	$K_2$	$\beta$
	Benzene	Methanol	Water			
1	1.0	0.45	0.55	1.67	1.11	1.50
2	1.2	0.4	0.4	1.40	0.94	1.49
3	1.5	0.5	0.2	0.59	0.47	1.13
4	1.5	0.2	0.5	3.00	3.00	1.00

Table I gives clear evidence that the diastereomers are separable by counter-current distribution. The number of transfers necessary to achieve the separation decreases with increasing  $\beta$ . Substantial amounts of methanol were necessary, while the ratios of benzene and water were varied widely with no loss of separability. The only drawback to the presence of a large amount of methanol was the possibility of emulsion formation, but this could be avoided by keeping the room temperature at 24° or less.

The principle advantage of counter-current distribution over the partition column is reproducibility of the separation. Counter-current distribution separation can be achieved by a single run whereas the partition column separation requires the mixture to be dried and re-chromatographed.

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