acid with 2,2,2-trifluoroethyl iodide.⁵ The acid chloride was formed by stirring the acid overnight in excess thionyl chloride. Excess reagent was removed under vacuum and the residue distilled to give 90-100% of acid chloride 14a as a pale yellow liquid, bp 46-48 °C (10 mm).

7-[[2-(2,2,2-Trifluoroethyl)sulfinyl]acetamido]cephalosporins (19, 22, 25). Triethylamine was added dropwise to a stirred suspension of 10 mmol of the appropriate 7-amino-3-cephem-4-carboxylic acid in 50 ml of dry DMF until solution was complete. The activated ester (10 mmol) was added in one portion and the resulting solution was stirred at room temperature for 1.5 h. The DMF was evaporated and the residue partitioned between 150 ml of EtOAc and 150 ml of H₂O. The pH was adjusted to 6.8 and the organic extract separated and discarded. The aqueous phase was layered with fresh EtOAc and adjusted to pH 2.0 with 3 N HCl. An emulsion usually formed which was broken by filtration through a pad of Celite. The filtrate layers were separated and the aqueous phase was extracted twice more with EtOAc. The combined extracts were dried and evaporated to give the cephalosporin. If the cephalosporin was not solid, it was dissolved in CH₃OH and titrated to pH 7.0 with 5% NaOCH₃ in CH₃OH. Et₂O was added dropwise with rapid stirring to precipitate the sodium salt of the cephalosporin which was collected and dried under vacuum.

7-[[2-(2,2,2-Trifluoroethyl)sulfonyl]acetamido]cephalosporins (20, 23, 26). A solution of 10 mmol of the *tert*-butyl 7-amino-3-cephem-4-carboxylate, 10 mmol of 2-[(2,2,2-trifluoroethyl)sulfonyl]acetic acid, and 10 mmol of DCC in 150 ml of dry THF was stirred at room temperature overnight. The precipitated urea was removed by filtration and the filtrate evaporated to a gum. This was dissolved in a solution of 20 ml of TFA and 20 ml of *m*-dimethoxybenzene and stirred at room temperature for 2 h. It was added dropwise to 300 ml of rapidly stirred ether and the resulting precipitate collected, washed with ether, and dried. The cephalosporin was converted to its sodium salt by dissolving it in 20 ml of MeOH and adjusting the pH to 7.0 with 5% NaOCH₃ in CH₃OH. The product was precipitated by the dropwise addition of Et₂O. Note Added in Proof. Several other laboratories have recently reported studies on some of the chemical and biological properties of compound 11.9,10

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11,12-Secoprostaglandins. 1. Acylhydroxyalkanoic Acids and Related Compounds

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The synthesis is described of a series of acylhydroxyalkanoic acids which embody structural modifications of that class of secoprostaglandins which are formally derived from the natural substances by scission of the cyclopentane ring between carbon atoms 11 and 12. These analogues have been tested for their ability to stimulate cAMP formation in the mouse ovary, a characteristic action of the (E)-prostaglandins, and for their ability to bind to the rat lipocyte prostaglandin receptor. Certain members of the series that most closely resemble the prostaglandins in structure (e.g., 8-acetyl-12-hydroxyheptadecanoic acid) markedly stimulate cAMP formation at concentrations in the pharmacological range and show a significant affinity for the prostaglandin receptor. Conversely, these compounds are not substrates for prostaglandin 15-hydroxydehydrogenase which catalyzes a major reaction in the biological deactivation of the prostaglandins.

A practical objective of prostaglandin analogue research is the development of a group of compounds with adequate metabolic stability and differing tissue specificities so that the numerous biological actions of the prostaglandins¹ can, in effect, be separated and applied in the treatment of various diseases. Our work in this field has centered on compounds that may be termed 11,12-secoprostaglandins since they are formally derived from the prostaglandins by cleavage of the C-11 to C-12 bond of the cyclopentane ring. Ring opening of prostaglandin E_1 in this manner gives, for example, a branched chain alkanoic acid 8-(R)-(3-hydroxypropionyl)-12(S)-hydroxy-10-transheptadecenoic acid (1).

During studies on the synthesis of 11,12-secoprostaglandins such as 1, it was discovered that the simpler analogue, 8-acetyl-12-hydroxyheptadecanoic acid (2a), possesses a number of the biological actions of the natural prostaglandins. The synthesis and biological examination





of acylhydroxyalkanoic acids related to 2a are the subject of this first paper of a series on the 11,12-secoprostaglandins.²

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Chemistry. Acylhydroxyalkanoic acids and derived

prepared as shown in Scheme I. tert-Butyl acetoacetate was alkylated with ethyl 7-bromoheptanoate to give dicarboxylic ester 6. This ester was alkylated with the array of acetoxy-substituted alkyl halides R'-hal (7a-i) and the resulting esters were heated with acid to effect elimination and decarboxylation and yield esters 9. Saponification of

preparation of acylhydroxy acids 2j-n which contain allylic and proparglyic hydroxy groups and thus readily undergo acid-induced dehydration. In Scheme II, ethyl acetoacetate was alkylated sequentially with ethyl 7-bromoheptanoate and the unsaturated acetoxyalkyl halides 7j-n to give dicarboxylic esters 11 which were saponified and decarboxylated to yield 2j-n.

The preparation of 8-propionyl-12-hydroxyheptadecanoic acid (3) is outlined in Scheme III. Sequential alkylation of di-tert-butyl malonate with ethyl 7-bromoheptanoate and 1-chloro-4-acetoxynonane (7a) gave the tricarboxylic ester 12 which was heated with acid to effect elimination and decarboxylation and yield nonanedioic acid half-ester 13. Reaction of 13 with thionyl chloride gave the acid chloride 14. This compound reacted smoothly with diethylcadmium. The resulting acetoxy keto ester was saponified to yield 3.





Futher compounds were prepared by reactions on these primary products. The acetylenic compounds 2l-n were hydrogenated to yield saturated chain acids 2o-q, respectively. Sodium borohydride reduction of 2a gave the 8-(1-hydroxyethyl) derivative 5; chromic acid oxidation of 2a gave the 12-keto derivative 2s. 8-Hydroxymethyl-12-hydroxyheptadecanoic acid (4) was obtained by NaBH₄ reduction of the acid chloride 14 (Scheme III).

The oxymercuration-demercuration reaction³ sequence was used in the preparation of 8-acetyl-12-methyl-12hydroxyheptadecanoic acid ($2\mathbf{r}$) (Scheme IV). Addition of mercuric acetate to the double bond of the olefinic keto ester 16 followed by demercuration with NaBH₄ gave the tertiary alcohol 17 which was saponified to yield $2\mathbf{r}$.

The final products and intermediate esters 8, 9, 11, 12, 16, and 17 are viscous oils that cannot be purified by distillation. The products and esters 9 and 17 were purified by column chromatography on silica gel. These substances retain solvents tenaciously and samples suitable for analysis and biological testing can be obtained only by being heated in high vacuum for long periods. For this reason, analytical data have been obtained, with few exceptions, only on the final product acids. The structures of the enumerated intermediates are supported by their NMR and ir spectra and the purity of chromatographed Scheme III



intermediates by thin-layer chromatographic evidence.

Where racemic alkylating agents R'-hal are used, the products 2 must consist of four stereoisomers in equal parts. Separation of isomers was not attempted except in the case of 2a. This one compound of the group, after long standing, deposited about half its weight of crystalline material, mp 53-55.5 °C, which could be efficiently separated. That this crystalline material is one of the two racemic forms of 2a can be seen by examination of the ¹H NMR spectra (CDCl₃) with and without the shift reagent $Eu(fod)_{3}$.⁴ The acetylmethyl protons of the total stereomixture 2a resonate as a sharp singlet at δ 2.12. Addition of Eu(fod)₃ shifts this signal downfield and splits it into two well-separated singlets of equal area. The corresponding signal of the crystalline fraction of 2a, originally also at δ 2.12, is shifted downfield by Eu(fod)₃ but remains one singlet with a chemical shift at the position of the

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Table I. Acylhydroxyalkanoic Acids



| | | | | 3. | Lipocvte | | | |
|-------------------------------|---|-----------------|---------|--|--|-----|---------------------------------------|------------------------|
| | | | | _ | Mouse ovary PG assay, fold increase in cAMP | | receptor binding, µg equiv to 1 | |
| No. | R | Yield, % | R_f^a | Formula ^b | 10 ¹ | 25' | 100' | ng of PGE ₁ |
| 2a | ОН | 26 ^c | 0.44 | $C_{19}H_{34}O_{4}$ | 11 | 14 | 23 | 1.7 |
| $\alpha \cdot 2 \mathbf{a}^d$ | | е | 0.44 | $C_{19}H_{36}O_{4}$ | 7 | 19 | 23 | |
| 2b | | 17 ^c | 0.46 | $C_{14}H_{26}O_{4}$ | | 1 | 1 | 450 |
| 2c | | 22 ^c | 0.46 | $C_{15}H_{28}O_{4}$ | | 1 | 1 | >100 |
| 2d | Он | 22 ^c | 0.56 | $C_{21}H_{40}O_{4}$ | 19 | 37 | 39 | 6.6 |
| 2e | | 18 ^c | 0.47 | $C_{19}H_{36}O_4$ | 5 | | 11 | 41 |
| 2 f | | 41 ^c | 0.51 | $C_{_{19}}H_{_{36}}O_{_4}$ | 9 | 9 | 20 | 2.1 |
| 2g | | 37 <i>°</i> | 0.58 | $C_{19}H_{36}O_{4}$ | 4 | 4 | 8 | 65 |
| 2 h | ОН | 14 ^c | 0.64 | $C_{19}H_{36}O_{4}$ | 1 | 1 | 1 | >125 |
| 2 i | C ₆ H ₅ | 30 ^c | 0.49 | $C_{22}H_{34}O_{4}$ | 1 | 1 | 2 | |
| 2 j | | 31^{f} | 0.53 | $C_{19}H_{34}O_4$ | 2 | 8 | 14 | 3.2 |
| 2 k | | 36 ^f | 0.47 | $C_{19}H_{32}O_4$ | 2 | 4 | 7 | |
| 21 ^g | | 40 ^f | 0.47 | $C_{19}H_{32}O_{4}$ | 2 | 3 | 3 | |
| $2\mathbf{m}^h$ | CH | 49 ^f | 0.47 | C ₁₉ H ₃₂ O ₄ | 3 | 7 | | |
| 2n | но | 49 ^f | 0.44 | $C_{19}H_{30}O_{4}$ | | 1 | 1 | |
| 20 | U OH | е | 0.44 | $C_{19}H_{36}O_4$ | 5 | 12 | 19 | 2.1 |
| 2p | Страна С Страна С С С С С С С С С С С С С С С С С С | е | 0.44 | $C_{19}H_{36}O_{4}$ | 19 | 25 | 29 | 3.2 |
| 2 q | но | е | 0.46 | C 19H 34O4 | 1 | 3 | 4 | >125 |
| 2r | но | е | 0.47 | $C_{20}H_{38}O_{4}$ | 4 | 9 | 11 | 0.4 |
| 2s | | е | 0.64 | $C_{19}H_{34}O_4$ | 2 | 4 | 9 | 7 |

Table I (Continued)

| | | | | | Mouse ovary PG assay, fold increase in cAMP | | | Lipocyte receptor binding, µg equiv to 1 |
|-----|-----|----------|-------------|-------------------------|--|-----------------|------------------|---|
| No. | R | Yield, % | R_{f}^{a} | Formula ^b | 10^l | 25 ¹ | 100 ^l | ng of PGE |
| 3 | ° – | е | 0.58 | $C_{20}H_{38}O_4^{i}$ | 6 | 17 | 26 | 2.4 |
| 4 | но | е | 0.30 | $C_{18}H_{36}O_4{}^j$ | 8 | 9 | 18 | 4.6 |
| 5 | OH | е | 0.30 | $C_{19}H_{38}O_4^{\ k}$ | 15 | 25 | 33 | 2.5 |

^a Determined on SiO₂ plates with CHCl₃-CH₃OH-AcOH (95:4:1). ^b All compounds were analyzed for C and H. Analytical results were within 0.4% of the theoretical values except where noted. ^c Overall yield from diester 6 (Scheme I). ^d Racemate, mp 53-55.5 °C. ^e See Experimental Section. ^f Overall yield from diester 10 (Scheme II). ^g $[\alpha]^{26}D + 2.18^{\circ}$ (c 3.85, CHCl₃). ^h $[\alpha]^{26}D - 1.94^{\circ}$ (c 3.45, CHCl₃). ⁱ H: calcd, 11.18; found, 11.69. ^j C: calcd, 68.31; found, 68.72. ^k H: calcd, 11.59; found, 12.03. ^l Concentration in μ g/ml.

Scheme V



upfield singlet of 2a. The fraction of 2a that remains an oil shows two singlets with the shift reagent, the downfield one predominating (~4:1). The crystalline racemate is designated α -2a in Table I.

Another separation of isomer pairs of 2a was achieved when 8-acetyl-12(R)-hydroxyheptadecanoic acid (2o) and 8-acetyl-12(S)-hydroxyheptadecanoic acid (2p) were synthesized. Scheme II was employed. The alkylating agents R'-hal were the R and S enantiomers of 1bromo-4-acetoxy-2-nonyne. The acetylenic epimer pairs 21 and 2m were obtained. Hydrogenation of these gave 20 and 2p.

Further separation into single stereoisomers was not attempted since the C-8 chiral center is configurationally labile (through enolization), making the configurational integrity of individual stereoisomers and racemic pairs uncertain. At any rate, with a racemate and the two epimeric pairs available, an estimate could be made of the dependence of biological activity on configuration in this key compound.

The various preparations of alkylating agents 7 are described in the Experimental Section. Two of the more broadly applied processes are shown in Schemes V and VI. The synthesis of 1-chloro-4-acetoxynonane (7a, Scheme V) exemplifies the process used to prepare 7a,d-g,i. The initial step, a Grignard reaction on chloronitriles, gave the intermediate chloro ketones in only modest yields (20-30%). The synthesis of 1-bromo-4-acetoxy-2-nonyne (7k, Scheme VI) exemplifies the process used to prepare propargylic bromides 7k-n. The final step was an efficient cyanogen bromide cleavage of an acetylenic Mannich amine.

Biological Activity. The prostaglandins of the E series have been shown to raise cAMP levels in cells of many types.⁵ The dose-related stimulation by PGE₁ of cAMP formation in the mouse ovary is the basis for the primary



assay used in these laboratories for the detection and measurement of prostaglandin-like activity.⁶ In this assay, described in detail in the Experimental Section, mouse ovaries are first incubated with adenine-8-¹⁴C to allow formation of intracellular ATP-¹⁴C. Then, the test compound along with the phosphodiesterase inhibitor theophylline is added and incubation is continued. Reactions are finally terminated by the addition of trichloroacetic acid, and cAMP-¹⁴C is isolated from the ovaries and measured. Results are expressed in this paper as fold increases in cAMP formation obtained by dividing the cAMP levels in treated ovaries by those levels found in untreated ovaries.

The effectiveness of 8-acetyl-12-hydroxyheptadecanoic acid (2a) in stimulating cAMP formation is compared with that of PGE₁, PGE₂, and tetrahydro-PGA₁ in Table II. Analogue 2a is seen to raise cAMP levels markedly at pharmacologically attainable concentrations, although these concentrations are about 1000 times the concentrations of PGE₁ required for similar effects. The related ricinoleic acid is without effect except at the highest concentrations.

It is important to demonstrate that the secoprostaglandins not only possess a characteristic action of the natural prostaglandins but that they can interact with prostaglandin receptors. Without the ability to so interact, these substances can hardly be described as prostaglandin analogues in any biological sense. A prostaglandin receptor binding assay has been devised in these laboratories that employs a binding fraction prepared from rat lipocytes.⁷ In this assay, the test compound is allowed to compete with tritiated PGE₁ for binding to the receptors. Results are here expressed as nanograms of test compound equivalent to 1 ng of cold PGE₁ in displacing tritiated PGE₁ from

| | Mouse ovary PG assay, fold increase in cAMP | | | | | | | | Lipocyte receptor binding, ng equiv to 1 | |
|--------------------------------------|---|------------|------------------|------------------|-----------------|--------|--------|------------------|---|---------------|
| | 0.01 ^a | 0.05^{a} | 0.1 ^a | 1.0 ^a | 10 ^a | 25^a | 50^a | 100 ^a | 200^{a} | ng of PGE_1 |
| PGE ₁ PGE ₂ | 8 14 | 29 35 | 25 57 | 54 75 | 80 | | | | | 1 1.4 |
| 502H | | | | 10 | 25 | 26 | 19 | | | 10 |
| о́- 2а | | | | 2 | 11 | 14 | 16 | 23 | 16 | 1700 |
| ricipoleic acid | | | | | | | 2 | 2 | 1 | |

^{*a*} Concentration in μ g/ml.

Table II

binding sites. These data, also presented in Table II, show that 2a appears to have weak but real affinity for the lipocyte PG receptor. The roughly 1000-fold loss in receptor affinity in going from PGE₁ to 2a parallels the 1000-fold loss of potency of 2a toward cAMP stimulation in the mouse ovary.

With a relationship established between the activity of our key analogue 2a and those of the natural (E)-prostaglandins, we may turn to the data obtained by evaluating the entire series of acylhydroxyalkanoic acids for their ability to stimulate cAMP formation and to bind to the lipocyte prostaglandin receptor. These data are presented in Table I. The relation between structure and activity in this series will be discussed by noting the changes in activity caused by structural modification of 2a.

The length and character of the hydrocarbon terminus are important determinants of activity. Activity is abolished by removal of the terminal 5- or 4-carbon atoms (to give 2c and 2b, respectively) and by replacement of the terminal 3-carbon atoms by phenyl (2i). Attachment of C-17 to the hydroxy-bearing C-12 to form a cyclohexane ring (2q) reduces activity to a low level. Addition of a 12-methyl group to give tertiary alcohol 2r significantly reduces activity.

At this point, it should be noted that the acylhydroxyalkanoic acids are not substrates for the enzyme prostaglandin 15-hydroxydehydrogenase which catalyzes the metabolic deactivation of the natural prostaglandins. Thus, addition of a 12-methyl group in this series is without the significance of the analogous addition of a 15-methyl group in the prostaglandin series which blocks the enzymatic dehydrogenation of the 15-position secondary alcohol function.

Activity is also highly dependent on the position of the hydroxy group. Activity is diminished when the hydroxy group is placed on the 11-carbon atom (2e). Activity is largely retained in the 13-OH isomer of 2a (2f) but is strongly reduced in the 14-OH isomer 2g and lost in the 17-OH isomer 2h.

Introduction of a trans-10,11 double bond which corresponds to the prostaglandin 13,14 double bond markedly reduces activity (2j). Introduction of an acetylenic bond in the same position (2k) reduces activity further.

Reduction of the ketone function in 2a to give the 8-(1-hydroxyethyl) derivative 5 significantly increases activity. Oxidation of 2a to give the 12-oxo analogue 2s markedly reduces activity—consistent with the effect of the similar oxidation (metabolic dehydrogenation) in the prostaglandin series. Lengthening the acetyl group of 2a to propionyl (3) did not affect activity.

The compounds compared thus far have been total mixtures of stereoisomers. The dependence of activity on configuration of the chiral centers of 8-acetyl-12hydroxyheptadecanoic acid can be assessed by comparing the cAMP stimulatory activities of the total stereomixture **2a**, the crystalline racemate, and the 12(R) and 12(S)epimeric pairs. The activity of the crystalline racemate (and, inferentially, of the uncrystallized racemate) does not differ significantly from that of 2a. Both epimeric pairs are active; however, the 12(S) pair in which the configuration of the hydroxy-bearing C-12 is the same as that of C-15 in the prostaglandins gives cAMP levels that are roughly double those produced by the 12(R) pair. The stereoselectivity of this action is nevertheless slight relative to the high order of stereoselectivity associated with many of the actions of the prostaglandins. This loss of stereoselectivity undoubtedly is connected with the loss of structural rigidity that attends the cutting of the cyclopentane ring of the natural substances.

The receptor binding data for this series correlate well with the cAMP stimulatory data. The values for relative receptor affinity of all active compounds cluster closely around that of 2a; inactive compounds show negligible affinity for the lipocyte receptor (e.g., 2b,c,h).

The evaluation of the acylhydroxyalkanoic acids in vivo is in progress in these laboratories. Complete results will be published elsewhere. It may suffice to state here that a number of these analogues which have shown prostaglandin-like activity in vitro and affinity for the prostaglandin receptor have also shown some, but not all, of the characteristic actions of the prostaglandins in whole animals.

For example, 2a possesses renal vasodilatory activity which can be demonstrated when renal blood flow is measured electromagnetically. Intravenous infusion of 0.5 mg/kg/min of 2a in anesthetized dogs gives a maximum increase in renal blood flow of 92 ml/min above the control value of 206 ml/min (measurements in single kidney). PGE₁ produces renal vasodilation but only on infusion into the renal artery when its metabolic inactivation is minimized.

Compound 2a inhibits collagen-induced platelet aggregation when administered orally to guinea pigs, ED_{50} 6.5 mg/kg. The ED_{50} of PGE_1 (not active by oral administration) is 0.02 mg/kg ip.

Experimental Section

Chemical. Melting points were taken in open capillary tubes

and are uncorrected as are boiling points. ¹H NMR spectra were obtained in $CDCl_3$ on a Varian A-60A spectrometer. Chemical shifts are reported as parts per million relative to Me₄Si as an internal standard. Optical rotations were measured with a Perkin-Elmer 141 polarimeter.

Column chromatography was carried out on E. Merck's silica gel 60, particle size 0.063–0.20 mm. Thin-layer chromatography (TLC) was used to monitor column fractions and to establish purity of products. It was performed on Analtech silica gel GF plates (thickness 250 μ). Spots were located with iodine vapor. A standard solvent system was used for TLC of all acid products (Table I) consisting of CHCl₃–CH₃OH–HOAc (95:4:1).

Chromatographed compounds were prepared for analysis and biological testing by being heated at 100 °C in oil pump vacuum for 4–6 h in order to remove the last traces of solvents. When analyses are indicated only by the symbols of the elements, the analytical results obtained for these elements are within 0.4% of the theoretical values.

Ethyl 8-tert-Butoxycarbonyl-9-oxodecanoate (6). tert-Butyl acetoacetate (126 g, 0.80 mol) was added during 80 min to a stirred suspension of NaH (21.1 g, 0.88 mol) in benzene (400 ml) and DMF (400 ml). The mixture was stirred for an additional 30 min. Ethyl 7-bromoheptanoate (208 g, 0.88 mol) was then added during 30 min and the mixture heated at 100 °C for 3.5 h. The mixture was cooled and treated with 1600 ml of water. The organic layer was separated, diluted with ether, and dried over MgSO₄. Vacuum distillation gave 158 g (63%) of 6: yellow oil; bp 175–177 °C (0.5 mm). Anal. (C₁₇H₃₀O₅) C; H: calcd, 9.62; found, 10.17.

Diethyl 2-Acetylnonanedioate (10). This compound was prepared analogously from ethyl acetoacetate and ethyl 7bromoheptanoate in 70% yield: bp 155–157 °C (0.5 mm). Anal. $(C_{15}H_{26}O_5)$ C, H.

1-Chloro-4-acetoxybutane $(7b)^8$ and 1-chloro-4-acetoxypentane $(7c)^9$ were prepared by published procedures. The preparation of 1-bromo-9-acetoxynonane (7h) was described recently;¹⁰ 7h has bp 126–127 °C (0.4 mm). Anal. (C₁₁H₂₁BrO₃) C, H.

1-Chloro-4-acetoxynonane (7a). (a) 1-Chloro-4-nonanone. 4-Chlorobutyronitrile (155 g, 1.5 mol) was added during 1 h to the Grignard reagent prepared from 1-bromopentane (227 g, 1.5 mol) and Mg (36.5 g, 1.5 g-atoms) in ether (1 l.). After an additional hour of stirring, the mixture was poured into finely crushed ice (1 kg) and concentrated hydrochloric acid (750 ml). The ether layer was separated and discarded. The aqueous solution was heated 1 h on a steam bath. The ketone which separated as an oil was taken up in ether, dried over MgSO₄, and distilled to yield 69.0 g (26%) of 1-chloro-4-nonanone: colorless oil; bp 115-117 °C (14 mm). Anal. (C₉H₁₇ClO) C, H.

(b) 1-Chloro-4-nonanol. 1-Chloro-4-nonanone (61.4 g, 0.35 mol) was added during 1 h to a suspension of NaBH₄ (6.5 g, 0.17 mol) in ethanol (310 ml) in which 1.3 g of NaOH had been dissolved. The temperature was kept at 45-50 °C. After an additional hour of stirring, the mixture was acidified with concentrated hydrochloric acid and the ethanol distilled at reduced pressure. The residue was treated with water and the oily product taken up in ether and dried over MgSO₄. Evaporation of ether left the product as a yellow residual oil which weighed 58.8 g: ir 3400 cm⁻¹ (OH).

(c) 1-Chloro-4-acetoxynonane (7a). A mixture of crude 1-chloro-4-nonanol (58.8 g, 0.33 mol) and Ac₂O (67.3 g, 0.66 mol) was heated at 95 °C for 1.5 h and then distilled at reduced pressure to yield 46.5 g (64%) (14% overall from 1-bromopentane) of 7a: bp 130-133 °C (14 mm); NMR δ 0.89 (3 H, t, CH₃), 2.02 (3 H, s, CH₃COO), 3.53 (2 H, t, CH₂Cl), 4.89 (1 H, m, HCOAc). Anal. (C₁₁H₂₁ClO₂) C, H.

1-Chloro-5-acetoxynonane (7f). This compound was prepared by a three-step process analogous to that used for 7a beginning with 1-bromobutane and 5-chlorovaleronitrile: overall yield 22%; bp (7f) 130-134 °C (13 mm). Anal. ($C_{11}H_{21}ClO_2$) C, H.

1-Bromo-6-acetoxynonane (7g). This compound was prepared analogously to 7a beginning with 1-bromopropane and 6-bromohexanenitrile: overall yield 14%; bp (7g) 142–145 °C (13 mm); ¹H NMR δ 3.45 (2 H, t, CH₂Br). Anal. (C₁₁H₂₁BrO₂) H; C: calcd, 49.82; found, 50.33.

1-Chloro-4-acetoxyundecane (7d). This compound was prepared analogously to 7a beginning with 1-bromoheptane and

4-chlorobutyronitrile: overall yield 9%; bp 155–158 °C (15 mm). Anal. $(C_{13}H_{25}ClO_2)$ C, H.

1-Chloro-4-acetoxy-6-phenylhexane (7i). This compound was prepared analogously to 7a beginning with phenethyl bromide and 4-chlorobutyronitrile: overall yield 24%; bp 185–193 °C (15 mm). Anal. ($C_{14}H_{19}ClO_2$) C, H.

1-Chloro-3-acetoxynonane (7e). 3-Chloropropanal (37.4 g, 0.40 mol) was added during 1 h to the Grignard reagent prepared from 1-bromohexane (73.9 g, 0.46 mol) and Mg (11.0 g, 0.46 mol) in Et₂O (200 ml). After 1 h additional reaction time, the mixture was worked up in the standard manner and the product distilled to yield 25.0 g (35%) of 1-chloro-3-nonanol, bp 123-126 °C (14 mm). A mixture of this alcohol (25 g, 0.14 mol) and Ac₂O (28.6 g, 0.28 mol) was heated at 95 °C for 15 h and then distilled to yield 26.8 g (87%) of 7e, bp 133-135 °C (14 mm). Anal. (C₁₁-H₂₁ClO₂) C, H.

(E)-1-Bromo-4-acetoxy-2-nonene (7j). A mixture of (E)-4-acetoxy-2-nonene¹¹ (73.5 g, 0.4 mol), N-bromosuccinimide (80.0 g, 0.45 mol), and CCl₄ (500 ml) was boiled under reflux for 3 h. The mixture was cooled and succinimide filtered off. The solution was washed with dilute NaHCO₃ solution and dried over Na₂SO₄. The solvent was evaporated and the residual oil distilled to yield 62 g (59%) of crude 7j, bp 110–112 °C (0.1 mm). For further purification, 20 g of this product was chromatographed in benzene on a column containing 275 g of silica gel. 7j (11 g) was obtained as a colorless oil: homogeneous on TLC (benzene) R_f 0.47; NMR δ 2.03 (3 H, s, CH₃COO), 3.87 (2 H, d, J = 6 Hz, CH₂Br), 5.20 (1 H, m, HCO), 5.6–5.9 (2 H, m, vinyl H). Anal. (C₁₁H₁₉BrO₂) H; C: calcd, 50.20; found, 49.69.

1-Bromo-4-acetoxy-2-nonyne (7k). (a) 3-Acetoxy-1-octyne. Acetic anhydride was added dropwise with stirring to a solution of 1-octyn-3-ol (100 g, 0.794 mol) in pyridine (79 g, 1.0 mol) during 1 h. The temperature rose to 45 °C. The solution was heated at 55 °C for 1 h and then cooled and poured into 300 ml of ice-cold 5% hydrochloric acid. The separated oil was taken up in ether, washed with water, dried over Na₂SO₄, and distilled to yield 106 g (80%) of product, bp 91–92 °C (15 mm). Anal. (C₁₀H₁₆O₂) H; C: calcd, 71.39; found, 71.89.

(b) 1-Diethylamino-4-acetoxy-2-nonyne. A mixture of 3-acetoxy-1-octyne (58.5 g, 0.35 mol), diethylamine (28.5 g, 0.39 mol), paraformaldehyde (13.8 g, 0.46 mol), and p-dioxane (60 ml) was heated at 95 °C under a reflux condenser for 17 h. The resulting solution was cooled, diluted with ether, and extracted with 300 ml of 5% hydrochloric acid. The acidic aqueous solution was made basic with 10% NaOH solution. The liberated amine was taken up in ether, washed with water, dried over Na₂SO₄, and distilled to yield 73.1 g (89%) of product, bp 103-109 °C (0.3 mm). Anal. (C₁₅H₂₇NO₂) C, H, N.

(c) 1-Bromo-4-acetoxy-2-nonyne (7k). A solution of 1-diethylamino-4-acetoxy-2-nonyne (50.6 g, 0.20 mol) and BrCN (21.2 g, 0.20 mol) in ether (250 ml) was let stand at 27 °C for 18 h. The solution was washed with 5% hydrochloric acid and water and dried over Na₂SO₄. The ether was evaporated and the residual oil distilled. After a forerun of diethylcyanamide, there was collected 34.1 g (65%) of 7k, bp 97–105 °C (0.2 mm). Anal. ($C_{11}H_{17}BrO_2$) C, H.

(4*R*)-1-Bromo-4-acetoxy-2-nonyne (7l) was prepared analogously to 7k from (3R)-1-octyn-3-ol:¹¹ [α]²⁶D +6.10° (*c* 3.05, CHCl₃). Bromide 71 was obtained in 73% yield: bp 113–115 °C (0.4 mm); [α]²⁶D +75.4° (*c* 3.2, CHCl₃). The intermediates obtained were (a) (3*R*)-3-acetoxy-1-octyne [86%; bp 86–88 °C (13 mm); [α]²⁶D +70.0° (*c* 3.10, CHCl₃)] and (b) (4*R*)-1-diethylamino-4-acetoxy-2-nonyne [80%; bp 114–117 °C (1 mm); [α]²⁶D +74.0° (*c* 3.16, CHCl₃)].

(4S)-1-Bromo-4-acetoxy-2-nonyne (7m) was prepared analogously to 7k from (3S)-1-octyn-3-ol:^{12,13} [α]²⁶D -6.61° (c 3.3, CHCl₃), -20.2° (c 3.3, Et₂O). Bromide 7m was obtained in 60% yield: bp 103-109 °C (0.4 mm); [α]²⁶D -83.1° (c 3.7, CHCl₃). The intermediates obtained were (a) (3S)-3-acetoxy-1-octyne [88%; bp 90-91 °C (15 mm); [α]²⁶D -79.1° (c 3.0, CHCl₃)] and (b) (4S)-1-diethylamino-4-acetoxy-2-nonyne [84%; bp 119-121 °C (1 mm); [α]²⁶D -80.5° (c 3.3, CHCl₃)].

1-Acetoxy-1-(3-bromo-1-propynyl)cyclohexane (7n). (a) 1-Acetoxy-1-(3-diethylamino-1-propynyl)cyclohexane. A mixture of 1-acetoxy-1-ethynylcyclohexane (64.0 g, 0.385 mol), diethylamine (30.9 g, 0.42 mol), paraformaldehyde (15.0 g, 0.5 mol), CuCl (1.5 g), and dioxane (60 ml) was stirred while an initial exothermic reaction took place and subsided. The mixture was then heated at 95 °C for 1.5 h. The mixture was cooled, diluted with ether, and extracted with cold 5% hydrochloric acid. The acidic aqueous solution was made basic with 10% NaOH solution. The liberated amine was taken up in ether, dried over Na₂SO₄, and distilled to yield 72.7 g (75%) of nearly colorless oily product: bp 113-115 °C (0.15 mm); NMR δ 1.07 (6 H, t, CH₃CH₂), 2.02 (3 H, s, CH₃COO), 2.60 (4 H, q, CH₃CH₂), 3.52 (2 H, s, CH₂C \equiv). Anal. (C₁₅H₂₅NO₂) C, H; N: calcd, 5.57; found, 5.13.

(b) 1-Acetoxy-1-(3-bromo-1-propynyl)cyclohexane (7n). A solution of 1-acetoxy-1-(3-diethylamino-1-propynyl)cyclohexane (61 g, 0.24 mol) and BrCN (31.8 g, 0.30 mol) in ether (350 ml) was let stand at 25 °C for 18 h. The solution was washed with 5% hydrochloric acid and water, dried over Na₂SO₄, and distilled to yield 34.8 g (55%) of 7n, bp 114–120 °C (0.2 mm). Anal. ($C_{11}H_{15}BrO_2$) C, H.

Physical, analytical, and yield data for the following acylhydroxyalkanoic acids and congeners 2-5 are listed in Table I. The method used for the preparation of 2a-i (Scheme I) is exemplified by the preparation of 8-acetyl-12-hydroxyheptadecanoic acid (2a).

(a) Ethyl 8-Acetyl-8-tert-butoxycarbonyl-12-acetoxyheptadecanoate (8a). Ester 6 (20.4 g, 0.065 mol) was added during 30 min to a stirred suspension of NaH (1.7 g, 0.071 mol) in benzene (40 ml) and DMF (40 ml). After an additional hour, 7a (15.8 g, 0.072 mol) and KI (100 mg) were added and the mixture was heated at 100 °C for 66 h. The mixture was cooled and treated with 200 ml of water. The organic layer was separated, diluted with 220, washed with water, and dried over MgSO₄. Vacuum distillation of solvents left 32.0 g of crude 8a as an orange residual oil: NMR δ 0.90 (3 H, t, 17-CH₃), 1.45 [9 H, s, (CH₃)₃C], 2.02 (3 H, s, CH₃COO), 2,12 (3 H, s, CH₃CO), 4.13 (2 H, q), 4.84 (1 H, m, HCOAc).

(b) Ethyl 8-Acetyl-12-acetoxyheptadecanoate (9a). A solution of 8a (32 g, 0.064 mol) and p-toluenesulfonic acid (1.0 g) in toluene (110 ml) was boiled under reflux for 22 h. The solution was cooled, washed with saturated NaHCO₃ solution and water, and dried over Na₂SO₄. The solvent was distilled leaving 26.7 g of residual oil. Ester 9a was isolated by chromatography on 400 g of silica gel. There was obtained 9.6 g (38%) of 9a as a colorless oil showing one spot on TLC (1% CH₃OH in CHCl₃), R_f 0.38. Anal. (C₂₃H₄₂O₅) C, H.

(c) 8-Acetyl-12-hydroxyheptadecanoic Acid (2a). A solution of ester 9a (9.6 g, 0.024 mol) and NaOH (3.7 g, 0.092 mol) in water (17 ml) and CH₃OH (150 ml) was let stand at 25 °C for 72 h. Most of the MeOH was distilled and the residual solution was diluted with water and extracted with ether. The aqueous solution was acidified with concentrated hydrochloric acid. The precipitated product was taken up in ether and dried over MgSO₄. Evaporation of the solvent left 7.6 g of crude 2a which was chromatographed on 125 g of SiO₂ with 2% CH₃OH in CHCl₃ elution to yield 5.4 g (69%) of 2a, a colorless viscous oil, showing one spot on standard TLC: NMR δ 0.90 (3 H, t, 17-CH₃), 2.12 (3 H, s, CH₃CO), 3.64 (1 H, m, CHOH), 6.65 (2 H, s, OH and COOH).

Compound 2a deposited a crop of small crystals on standing at room temperature for 8 weeks. A 6.2-g sample was stirred with 5 ml of CH₃CN. The undissolved crystalline material was collected on a filter and washed with 2 ml of CH₃CN. There was obtained 1.4 g of colorless crystals, mp 53–55.5 °C, identical with stereomixture 2a in NMR spectrum and R_f (TLC).

The method used for the preparation of acylhydroxyalkanoic acids 2j-n (Scheme II) is exemplified by the preparation of 8-acetyl-12-hydroxy-10-heptadecynoic acid (2k).

(a) Diethyl 2-Acetyl-2-(4-acetoxy-2-nonyn-1-yl)nonanedioate (11k). Ester 10 (36.7 g, 0.128 mol) was added during 30 min to a stirred suspension of NaH (3.4 g, 0.14 mol) in benzene (65 ml) and DMF (65 ml). After 1 h of additional stirring, bromide 7k (36.7 g, 0.14 mol) was added and the mixture was heated at 100 °C for 1 h. The mixture was cooled and treated with 300 ml of water. The organic layer was separated, washed with water, and dried over Na₂SO₄. The solvent was evaporated to leave 59.8 g of crude 11k.

(b) 8-Acetyl-12-hydroxy-10-heptadecynoic Acid (2k). A solution of ester 11k (59.7 g, 0.128 mol) and NaOH (30 g, 0.75 mol) in water (200 ml) and CH₃OH (800 ml) was heated at 60

°C for 16 h. Most of the MeOH was then distilled at reduced pressure. The residue was dissolved in water and the solution acidified to congo red with concentrated hydrochloric acid. The separated acid was taken up in ether and dried over Na₂SO₄. Evaporation of the ether left 41.1 g of crude **2k** as a red viscous oil. It was purified by chromatography on 650 g of silica gel with 2% MeOH in CHCl₃ as eluent. There was obtained 15.0 g (36%) of **2k**, a yellow viscous oil, showing one spot on standard TLC: NMR δ 0.90 (3 H, t, 17-CH₃), 2.20 (3 H, s, CH₃CO), 4.37 (1 H, t, CHOH).

(12*R*)-8-Acetyl-12-hydroxyheptadecanoic Acid (20). (12*R*)-8-Acetyl-12-hydroxy-10-heptadecynoic acid (21) (25.8 g, 0.079 mol) was dissolved in a mixture of EtOAc (80 ml) and cyclohexane (160 ml) and hydrogenated over a 5% Pt on charcoal catalyst (3.5 g) in a Parr apparatus with an initial hydrogen pressure of 45 lb/in.². The uptake of the required 0.158 mol of hydrogen required 10 min. The catalyst was removed by filtration and the solvents were evaporated. The residual oily product was chromatographed on 400 g of silica gel with 2% CH₃OH in CHCl₃ elution. There was obtained 11.7 g (45%) of **21**: nearly colorless viscous oil; $[\alpha]^{26}$ D -0.79° (c 3.8, CHCl₃).

(12S)-8-Acetyl-12-hydroxyheptadecanoic Acid (2p). This compound was prepared analogously in 60% yield by hydrogenation of 2m: $[\alpha]^{26}D + 1.0^{\circ}$ (c 3.9, CHCl₃).

8-Acetyl-11-(1-hydroxycyclohexyl)-10-undecynoic Acid (2q). This compound was prepared analogously in 85% yield by hydrogenation of 2n.

8-(1-Hydroxyethyl)-12-hydroxyheptadecanoic Acid (5). Compound 2a (7.2 g, 0.022 mol) and NaBH₄ (0.76 g, 0.02 mol) were dissolved in a solution of NaOH (1.2 g, 0.03 mol) in water (80 ml). The resulting solution was allowed to stand at 27 °C for 20 h. It was then acidified with concentrated hydrochloric acid. The oily acid that separated was taken up in ether, dried over Na₂SO₄, and chromatographed on 120 g of silica gel with 4% CH₃OH in CHCl₃ elution. There was obtained 4.0 g (55%) of 5.

8-Acetyl-12-oxoheptadecanoic Acid (2s). A solution of 2a (9.8 g, 0.03 mol) in acetone (30 ml) was cooled to 5 °C and treated dropwise during 2 h with a solution prepared from CrO₃ (2.6 g, 0.026 mol), concentrated H₂SO₄ (2.1 ml), and water (7.5 ml). The solution was then diluted with water (250 ml). The oily layer was taken up in ether, washed with water, and dried over Na₂SO₄. Evaporation of ether left 9.1 g (93%) of 2s: yellowish oil; NMR δ 0.88 (3 H, t, 17-CH₃), 2.12 (3 H, s, CH₃CO), 2.38 (7 H, m, CHCO and various CH₂CO), 11.18 (1 H, s, COOH).

8-Propionyl-12-hydroxyheptadecanoic Acid (3) (Scheme III). (a) Di-tert-butyl (6-Ethoxycarbonylhexyl)malonate. Di-tert-butyl malonate (41.1 g, 0.19 mol) was added to a stirred suspension of NaH (5.0 g, 0.21 mol) in benzene (95 ml) and DMF (95 ml). Then ethyl 7-bromoheptanoate (49.8 g, 0.21 mol) was added during 30 min and the mixture was heated at 100 °C for 4.5 h. The mixture was cooled and treated with 500 ml of water. The organic layer was separated, diluted with ether, and dried over MgSO₄. The solvents were evaporated leaving 69.7 g of crude tricarboxylic ester.

(b) Di-tert-butyl 2-(4-Acetoxynonyl)-2-(6-ethoxycarbonylhexyl)malonate (12). The above ester (69.7 g, 0.187 mol) was alkylated in the same manner with NaH (5.0 g, 0.21 mol) and 7a (46.3 g, 0.21 mol). The mixture was heated at 100 °C for 42 h. Work-up gave 104.1 g of crude 12 as a residual oil.

(c) Ethyl 8-Carboxy-12-acetoxyheptadecanoate (13). A solution of 12 (104.1 g, 0.187 mol) and p-toluenesulfonic acid (3.3 g) in toluene (330 ml) was boiled under reflux for 9.5 h. The solution was worked up as in the preparation of 9a to yield 74.9 g of crude 13, a viscous red oil, which was chromatographed on 700 g of silica gel with 2% CH₃OH in CHCl₃ elution. There was obtained 46 g (62%) of 13 showing one spot, R_f 0.42, on TLC [CHCl₃-CH₃OH-AcOH (98:1:1)]. Anal. (C₂₂H₄₀O₆) C, H.

(d) Ethyl 8-Chlorocarbonyl-12-acetoxyheptadecanoate (14). A solution of 13 (12.0 g, 0.03 mol) and $SOCl_2$ (7.2 g, 0.06 mol) in benzene (50 ml) was boiled under reflux for 2.5 h. Volatile materials were removed on a rotary evaporator. The residual crude 14 weighed 12.5 g (100%): ir (neat) 1790 (COCl), 1730 cm⁻¹ (ester CO).

(e) Ethyl 8-Propionyl-12-acetoxyheptadecanoate. A solution of EtMgBr in ether (100 ml) was prepared from EtBr (5.5

g, 0.05 mol) and Mg (1.2 g, 0.05 mol). The solution was chilled to 0 °C and CdCl₂ (5.5 g, 0.03 mol) was added. The mixture was stirred 10 min without cooling and 30 min at reflux. Most of the ether was then allowed to distill off and benzene (100 ml) was added and then acid chloride 14 (12.5 g, 0.03 mol) during 20 min. The mixture was boiled under reflux for 2 h, then cooled, and treated with a 10% solution of H₂SO₄. The benzene layer was separated, dried over Na₂SO₄, and evaporated leaving the product as a residual oil which was chromatographed on 200 g of silica gel with CHCl₃ elution. There was obtained 6.2 g (50%) of the title ester showing one spot, R_f 0.23, on TLC (CHCl₃). Anal. (C₂₄H₄₄O₅) C, H.

(f) 8-Propionyl-12-hydroxyheptadecanoic Acid (3). A solution of the above ester (6.0 g, 0.146 mol) and NaOH (1.0 g, 0.025 mol) in water (10 ml) and MeOH (70 ml) was let stand at 27 °C for 24 h. Work-up as in the preparation of 2a gave 4.5 g of oily product which was chromatographed on 60 g of silica gel with 2% CH₃OH in CHCl₃ elution to yield 2.4 g (48%) of 3.

8-Hydroxymethyl-12-hydroxyheptadecanoic Acid (4). Acid chloride 14 (14.0 g. 0.335 mol) was added to a solution of NaBH₄ (2.7 g, 0.07 mol) in diglyme (75 ml). The exothermic reaction caused the temperature to rise to 55 °C. After 2 h, the solution was cooled in an ice bath and acidified with 10% hydrochloric acid. Water (250 ml) was then added and the oily product taken up in ether, washed with water, and dried over Na₂SO₄. The ether was evaporated to leave 12.2 g of crude ethyl 8-hydroxymethyl-12-hydroxyheptadecanoate. The ester was dissolved in a solution of NaOH (4.0 g, 0.1 mol) in water (20 ml) and MeOH (100 ml). The solution was allowed to stand for 64 h at 25 °C and then worked up as in the preparation of **2a** to obtain 6.6 g of crude 4. Column chromatography on 110 g of silica gel with 4% CH₃OH in CHCl₃ elution gave 3.7 g (35%) of purified 4: colorless viscous oil; NMR & 0.90 (3 H, t, 17-CH₃), 2.32 (2 H, t, CH₂COOH), 3.55 (3 H, m, CHOH and CH₂OH), 5.1 (3 H, br s, OH and COOH).

8-Acetyl-12-hydroxy-12-methylheptadecanoic Acid (2r) (Scheme IV). (a) Ethyl 8-Acetyl-8-tert-butoxycarbonyl-12-methyl-11-heptadecenoate (15). Ester 6 (81.4 g, 0.259 mol) was added during 30 min to a stirred suspension of NaH (6.8 g, 0.284 mol) in benzene (130 ml) and DMF (130 ml). After an additional hour, 1-bromo-4-methyl-3-nonene¹⁴ (62.2 g, 0.284 mol) was added and the mixture was heated at 100 °C for 20 h. Work-up as in the preparation of 8a gave 124.4 g of crude 15 as a red residual oil.

(b) Ethyl 8-Acetyl-12-methyl-11-heptadecenoate (16). A solution of 15 (124.4 g, 0.259 mol assumed) and *p*-toluenesulfonic acid in toluene (450 ml) was boiled under reflux for 21 h. Work-up as in the preparation of 9a gave 94.8 g of crude 16.

(c) Ethyl 8-Acetyl-12-hydroxy-12-methylheptadecanoate (17). Mercuric acetate (3.8 g, 0.012 mol) was dissolved in water (12 ml) and THF (20 ml) was added to give a suspension of a yellow solid. Ester 16 (4.2 g, 0.012 mol) in THF (20 ml) was added and the mixture was stirred at 27 °C for 24 h. After 6 h, the yellow solid had disappeared and a cloudy solution resulted. To this solution, a 3 M NaOH solution (12 ml) and then a 0.5 M solution of NaBH₄ in 3 M NaOH (12 ml) were added. Liquids were decanted from the precipitated mercury. The organic layer was taken up in ether, washed with water, and dried over Na₂SO₄. Evaporation of the ether left 4.4 g of 17 as a yellow oil. Column chromatography on 70 g of silica gel with CHCl₃ elution gave 2.9 g (65%) of 17 showing one spot, R_f 0.27, on TLC (1% CH₃OH in CHCl₃). Anal. (C₂₂H₄O₄) C, H.

(d) 8-Acetyl-12-hydroxy-12-methylheptadecanoic Acid (2r). Ester 17 (4.6 g, 0.0124 mol) and NaOH (1.0 g, 0.025 mol) were dissolved in a mixture of water (10 ml) and CH₃OH (50 ml) and the solution was let stand at 27 °C for 64 h. Work-up as in the preparation of 2a gave 3.5 g of oily product which was chromatographed on 60 g of silica gel with 2% CH₃OH in CHCl₃ elution. There was obtained 1.5 g (36%) of 2r: colorless viscous oil; NMR δ 0.90 (3 H, t, 17-CH₃), 1.13 (3 H, s, CH₃COH), 2.10 (3 H, s, CH₃CO), 6.80 (2 H, br s, OH and COOH). **Biological.** Mouse Ovary Prostaglandin Assay.⁶ Virgin female mice over 70 days old (Charles River CD-1) were killed and the ovaries dissected and denuded of adhering fatty tissue. Three ovaries were weighed (15–25 mg) and placed in 2 ml of aerated Krebs-Ringer phosphate buffer, pH 7.2, containing 1 μ Ci of adenine-8-¹⁴C. The tissues were incubated 1 h at 37 °C with moderate shaking to cause a pool of intracellular ATP-¹⁴C to accumulate.

The following additions were then made: 0.2 ml of 0.05 M theophylline in 0.15 M NaCl and the test compound in 0.1 ml of Me₂SO. The ovaries were again incubated at 37 °C for 30 min. The reactions were terminated by the addition of 0.4 ml of 10% trichloroacetic acid, and 50 μ l of a nucleotide mixture solution¹⁵ was added to facilitate recovery of the labeled nucleotides. The incubation mixture was transferred to a glass homogenizer and the ovarian tissue was homogenized into the acidified incubation solution. The homogenate was centrifuged 1000g for 5 min and the cAMP-¹⁴C was isolated from the supernatant fluid as described by Humes and co-workers¹⁴ including the final paper chromatography step.

Prostaglandin Receptor Binding Assay. Details of this assay have been published.⁷ Appropriate concentrations of the test compound were incubated with 0.4 ng of $[^{3}H]$ -PGE₁ and 125 μ g of the rat lipocyte binding preparation for 60 min at 37 °C. The amount of $[^{3}H]$ -PGE₁ associated with the binding preparation was determined as described in the reference. Duplicate experiments were run on each test compound at each of three concentrations.

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