Prostaglandins and Congeners. 14.[1] Synthesis and Bronchodilator Activity of *dl*-16,16-Trimethyleneprostaglandins

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The interesting bronchodilator activity of novel *dl*-16,16-trimethyleneprostaglandin congeners and their preparation via the conjugate addition of the appropriate vinyl lithiocuprate reagent to several cyclopentenones are described. Also discussed is the preparation of the key intermediate vinyl iodide **7**.

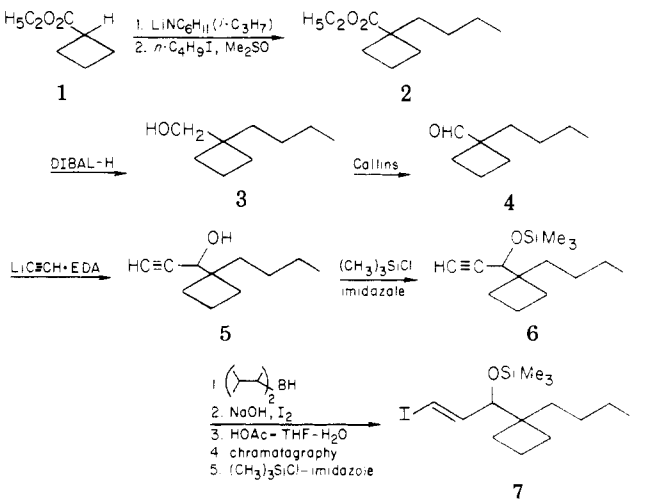
The prostaglandins, although short-acting, are very potent pharmacological agents with an extraordinarily broad biological profile.[2] Recent reports[3] indicate that C-15 metabolism apparently is effectively restricted in congeners bearing 16-alkyl substituents, and these compounds show a high degree of biological potency, at least as gastric acid secretion (GAS) inhibitors and antifertility agents. Furthermore, as GAS inhibitors they are effective for a prolonged period on oral administration.

Encouraged by these reports, but aware that the 16-alkyl congeners generally are characterized by poor therapeutic indices,[4] we have prepared a series of analogues possessing the unique trimethylene moiety at C-16 in the search for a compound with more selective activity. The synthetic scheme that we employed is based upon the convenient lithiocuprate conjugate addition of a fully elaborated β chain to several cyclopentenones with varying α chains according to the procedure of Sih and co-workers.[5] Herein we describe the synthesis of these novel *dl*-16,16-trimethylene congeners.[6]

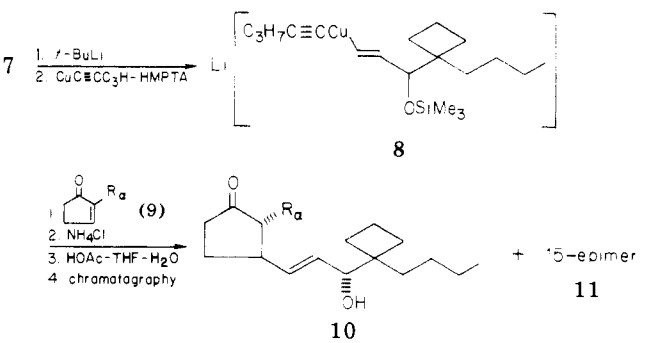
Chemistry. The preparation of the requisite precursor vinyl iodide **7** is shown in Scheme I. Alkylation[7] of the lithium enolate of commercially available ethyl cyclobutanecarboxylate (**1**) with *n*-butyl iodide furnished ester **2** which was reduced to the alcohol **3** by treatment with diisobutylaluminum hydride[8] (DIBAL-H). Initial attempts to reduce **2** directly to the corresponding aldehyde **4** with 1 equiv of DIBAL-H at −78 °C gave no detectable amount of **4**. Upon work-up, only a mixture of starting ester and alcohol **3** was isolated. Apparently the bulky trimethylene moiety induces a collapse of the intermediate aluminate reduction product to generate the aldehyde which then reacts with additional DIBAL-H. Collins oxidation[9] of alcohol **3** provided the aldehyde **4**, which was condensed with the lithium acetylide–ethylenediamine complex[10] (LiC≡CH·EDA) to give the propargylic alcohol **5**. After protection[11] of the hydroxyl function as the trimethylsilyl (Me_3Si) ether **6**, hydroboration–oxidation–iodination according to the procedure of the Syntex group,[12] followed by Me_3Si hydrolysis, chromatography, and reprotection, yielded (*E*)-1-iodo-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**7**).

The vinyl cuprate **8**, generated by sequential treatment of **7** with *tert*-butyllithium and copper(I) pentyne[13,14]–hexamethylphosphorous triamide[14] (HMPTA), was treated with cyclopentenones **9a**[15] and **9b**.[16] Acidic hydrolysis of the Me_3Si-protecting groups of the conjugate adducts, followed by dry column chromatography (DCC), furnished *dl*-11-deoxy-16,16-trimethyleneprostaglandin E_1 ethyl ester (**10a**) and its C-15 epimer **11a** and the *dl*-11-deoxy-16,16-trimethyleneprostaglandin E_2 epimers **10b** and **11b**, respectively. The conjugate addition sequence is illustrated in Scheme II.

Scheme I



Scheme II

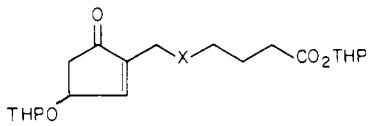


a, R_α = $-CH_2(CH_2)_5CO_2CH_2CH_3$
b, R_α = $-CH_2CH{=}CH(CH_2)_3CO_2CH_2CH_3$
c, R_α = $-CH_2(CH_2)_4CO_2CH_2CH_3$
d, R_α = $-CH_2(CH_2)_6CO_2CH_2CH_3$
e, R_α = $-CH_2(CH_2)_5CO_2CH_2CH_3$
f, R_α = $-CH_2(CH_2)_3C(CH_3)_2CH_2CO_2CH_2CH_3$
g, R_α = $-CH_2(CH_2)_3SCH_2CO_2CH_2CH_3$

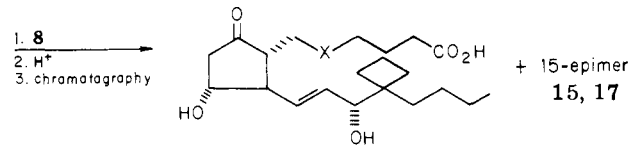
Similarly, cyclopentenones[15] **9c–e** provided analogues with varying α-chain length: the 2-nor (**10c** and **11c**), 1a-homo (**10d** and **11d**), and 1a,1b-dihomo (**10e** and **11e**) members of the *dl*-11-deoxy-16,16-trimethylene series. Also prepared were compounds resistant to fatty acid β-oxidation.[18] These were the 3,3-dimethyl (**10f** and **11f**) and 3-thia (**10g** and **11g**) derivatives obtained via cyclopentenones[15] **9f** and **9g**, respectively. Alkaline hydrolysis of these esters smoothly furnished the corresponding acids.

The 11-hydroxy analogues were prepared by the conjugate addition of vinyl cuprate **8** to the bis(tetrahydropyranyl) (THP) protected cyclopentenones **12**[19] and **13**.[20]

Acidic hydrolysis of the THP- and Me_3Si-protecting groups of the intermediate adducts, followed by DCC, furnished dl-16,16-trimethyleneprostaglandin E_1 (**14**) and dl-16,16-trimethyleneprostaglandin E_2 (**16**) and their 15-epimers, respectively. Also prepared by a completely analogous sequence was dl-20-nor-16,16-trimethyleneprostaglandin E_2 (**18**) and its 15-epimer **19**.



12, X = $-CH_2CH_2-$
13, X = -CH=CH- (cis)



14, X = $-CH_2CH_2-$
16, X = -CH=CH- (cis)

The conjugate addition yields usually ranged between 32 and 44% for the 11-deoxy congeners and were 22–23% in the 11-hydroxy series. The ratio of C-15 epimers[6] was approximately 60:40 (epi–nat), consistent with previous findings in these laboratories[18] wherein conjugate addition was effected with lithium alanate reagents.

Biology. The bronchodilator effect of these compounds was measured by a previously described procedure[21] by intravenous administration of candidate compounds to guinea pigs previously treated with the bronchoconstrictors serotonin, histamine, or acetylcholine (Table I). The 16,16-trimethylene derivatives **14** and **15** of dl-PGE_1 and dl-PGE_2 were very potent bronchodilators in this assay and gave results comparable to that observed with l-PGE_1. Even the 15-epimers were substantially active. It is also of interest that shortening the β chain in dl-16,16-trimethylene-20-norprostaglandin E_2 (**18**) did not affect the potency.

The 11-deoxy members of this series wherein the seven-atom E_1 or E_2 α chain was retained were relatively potent compounds although less active than the corresponding 11α-hydroxy derivatives, as might be expected. The 3-thia congener was somewhat less potent, but the 3,3-dimethyl analogue was relatively ineffective. Homologation of the α chain by one or two carbon atoms (**10d** acid and **10e** acid) was not consistent with bronchodilator activity, but abbreviating the chain by one atom did give an effective compound (**10c** acid), albeit one of diminished potency. In contrast to the 11α-hydroxy series the 11-deoxy-15-epimers were, with one exception (**11a** acid), relatively inactive.

When tested in the anesthetized dog by aerosol administration dl-16,16-trimethyleneprostaglandin E_1 (**14**) and dl-16,16-trimethyleneprostaglandin E_2 (**15**) inhibited at relatively low doses pilocarpine-induced bronchospasms.[23] In contrast to l-PGE_1 which was more potent but short-acting, these effects persisted for the duration of the experiments (1 h). Unfortunately, both 16,16-trimethylene derivatives produced a short-lived pulmonary hypertension at effective dose levels.

Experimental Section

All organometallic reactions were performed under an inert atmosphere of argon or nitrogen. Melting points were taken in open capillaries (Mel-Temp) and boiling points are uncorrected. All organic extracts were dried with anhydrous $MgSO_4$. Solvents were removed under reduced pressure using a Büchi evaporator. Infrared spectra were recorded with neat samples on a Perkin-Elmer Model 21 spectrophotometer. Nuclear magnetic resonance spectra were determined in $CDCl_3$ using Varian A-60 or HA-100D spectrophotometers. Chemical shifts are given in parts per million downfield from an internal $(CH_3)_4Si$ standard. Those analytical results indicated by symbol only were within ±0.4% of their calculated values. Ultraviolet spectra were obtained using a Cary recording spectrophotometer in the indicated solvent. Mass spectra were recorded on an AEI MS-9 at 70 eV. Only certain characteristic spectral data are presented for each compound.

Ethyl 2,2-Trimethylenehexanoate (2). To a stirred solution of 27.6 g (0.195 mol) of freshly distilled *N*-isopropylcyclohexylamine in 200 mL of dry THF cooled to −78 °C was added rapidly 96 mL (0.196 mol) of 2.04 M *n*-BuLi in hexane. To the resulting solution was added dropwise 25 g (0.195 mol) of ethyl cyclobutanecarboxylate (**1**). After 30 min the resulting solution was allowed to warm to ambient temperature and was transferred to a dropping funnel under N_2 and added dropwise during 75 min to a solution of 54 g (0.2935 mol) of *n*-butyl iodide in 100 mL of dry Me_2SO, maintaining the temperature at 16–20 °C. Stirring was continued for an additional 30 min. The separated salts were removed by filtration and the filtrate after concentration to a small volume was diluted with hexanes. This solution was washed with 2% HCl and brine and dried. After the evaporation of solvent the residual oil was distilled to give 14.6 g (41%) of **2**: bp 84–87 °C (10 mm); IR 2940 and 1725 cm^{-1}; NMR δ 4.15 (q, 2 H, $-OCH_2CH_3$, J = 7 Hz), 1.25 (t, 3 H, OCH_2CH_3, J = 7 Hz), and 0.89 ppm (t, 3 H, $-CH_2CH_3$). Anal. ($C_{11}H_{20}O_2$) H; C: calcd, 71.69; found, 71.16.

2,2-Trimethylenehexan-1-ol (3). To a stirred solution containing 20 g (0.109 mol) of ethyl 2,2-trimethylenehexanoate (**2**) in 100 mL of dry toluene under an argon atmosphere and cooled in an ice bath was added dropwise 250 mL (2 molar equiv) of 0.89 M diisobutylaluminum hydride in toluene. The resulting solution was stirred at ambient temperature for 2 h and then poured into excess iced 5% HCl. The organic phase was separated and washed with 5% HCl and brine. After drying it was taken to dryness to give 14.8 g (96%) of oil: bp 92–93 °C (10 mm); IR 3450 and 2900 cm^{-1}; NMR δ 3.52 (s, 2 H, $-CH_2OH$) and 0.90 ppm (t, 3 H, $-CH_2CH_3$, J = 7 Hz). Anal. ($C_9H_{18}O$) C, H.

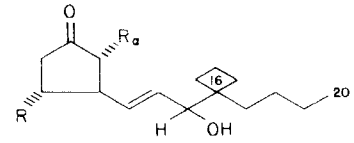
2,2-Trimethylenehexaldehyde (4). Chromium trioxide (61.5 g, 0.615 mol), dried in a vacuum desiccator over phosphorus pentoxide, was added to an ice-cold solution containing 97 g (1.226 mol) of dry pyridine in 1 L of dry CH_2Cl_2 and the deep red suspension was stirred for 45 min at ambient temperature. A solution of 14.5 g (0.102 mol) of 2,2-trimethylenehexan-1-ol (**3**) in 55 mL of dry CH_2Cl_2 was added all at once to the suspension. A black tarry deposit was formed immediately. After stirring at ambient temperature for 15 min, the solution was decanted from the tarry deposit which was then triturated four times with small portions of CH_2Cl_2. The combined extracts were washed twice each with ice-cold NaOH, ice-cold HCl, and finally brine, dried, and taken to dryness. Distillation of the residual oil gave 12.9 g (90%) of **4**: bp 69 °C (11 mm); IR 2740 and 1725 cm^{-1}.

The product was characterized as its 4′-phenylsemicarbazone, mp 97–98 °C. Anal. ($C_{16}H_{23}N_3O$) C, H, N.

4,4-Trimethylene-1-octyn-3-ol (5). To a solution of the lithium acetylide–ethylenediamine complex (9.4 g, 0.102 mol) in 90 mL of dry Me_2SO, cooled in an ice bath, was added 12.94 g (0.092 mol) of 2,2-trimethylenehexaldehyde (**4**) in 10 mL of Me_2SO dropwise at such a rate that the temperature was maintained at 20–25 °C. The solution was stirred at ambient temperature for 12 h and then poured into a mixture of ice-cold 2% HCl and ether. The ether layer was separated and the aqueous phase was extracted with ether. The combined ether extracts were washed with brine, dried, and evaporated. Distillation of the residual oil provided 13.53 g (88%) of **5**: bp 108–109 °C (13 mm); IR 3500, 3390, 2985, and 2125 cm^{-1}; NMR δ 4.31 [d, 1 H, $HC{\equiv}CCH(OH)-$, J = 2 Hz], 2.43 [d, 1 H, $HC{\equiv}CCH(OH)$, J = 2 Hz], and 0.95 ppm (t, 3 H, $-CH_2CH_3$, J = 7 Hz).

4,4-Trimethylene-3-trimethylsilyloxy-1-octyne (6). To a stirred solution of 5.3 g (31.9 mmol) of 4,4-trimethylene-1-octyn-3-ol (**5**) and 5.42 g (79.7 mmol) of imidazole in 32 mL of dry DMF, cooled in an ice bath and under an argon atmosphere, was added 4.35 g (40.0 mmol) of chlorotrimethylsilane. After stirring

Table I. *dl*-16,16-Trimethyleneprostaglandins and Their Bronchodilator Activity



Compound	R	R_α	Confign at C_{15}[a]	Yield,[b] %	Emp formula	Analyses[c]	Guinea pig bronchodilator assays,[d] ED_{50}, g/kg		
							Serotonin	Histamine	Acetylcholine
		l-PGE_1					1.07×10^{-6}	7.0×10^{-7}	3.3×10^{-6}
10a	H	$-(CH_2)_6CO_2C_2H_5$	N	25	$C_{25}H_{42}O_4$	C, H	409×10^{-6}	15.1×10^{-6}	
11a	H	$-(CH_2)_6CO_2C_2H_5$	E	38	$C_{25}H_{42}O_4$	C, H		32×10^{-6}	2.06×10^{-3}
10a acid	H	$-(CH_2)_6CO_2H$	N	92*	$C_{23}H_{38}O_4$	C, H	20.3×10^{-6}	2.4×10^{-6}	47.8×10^{-6}
11a acid	H	$-(CH_2)_6CO_2H$	E	93*	$C_{23}H_{38}O_4$	C, H	135×10^{-6}	13.9×10^{-6}	*e*
10b	H	$-CH_2CH{=}CH(CH_2)_3CO_2C_2H_5$	N	17 (1)	$C_{25}H_{40}O_4$	C, H		221×10^{-6}	
11b	H	$-CH_2CH{=}CH(CH_2)_3CO_2C_2H_5$	E	26 (1)	$C_{25}H_{40}O_4$	C, H	*e*	*e*	*e*
10b acid	H	$-CH_2CH{=}CH(CH_2)_3CO_2H$	N	90*	$C_{23}H_{36}O_4$	C, H	7.67×10^{-6}	2.20×10^{-6}	24.2×10^{-6}
11b acid	H	$-CH_2CH{=}CH(CH_2)_3CO_2H$	E	98*	$C_{23}H_{36}O_4$	C, H		*e*	*e*
10c	H	$(CH_2)_5CO_2C_2H_5$	N	11 (1)	$C_{24}H_{40}O_4$	*f*	*e*	*e*	*e*
11c	H	$(CH_2)_5CO_2C_2H_5$	E	3 (1)	$C_{24}H_{40}O_4$	H; C[g]			
10c acid	H	$(CH_2)_5CO_2H$	N	74*	$C_{22}H_{36}O_4$	*h*	32×10^{-6}	32×10^{-6}	320×10^{-6}
10d	H	$(CH_2)_7CO_2C_2H_5$	N	16 (4)	$C_{26}H_{44}O_4$	C, H	*e*	*e*	*e*
11d	H	$(CH_2)_7CO_2C_2H_5$	E	22 (4)	$C_{26}H_{44}O_4$	C, H	*e*	*e*	*e*
10d acid	H	$(CH_2)_7CO_2H$	N	76*	$C_{24}H_{40}O_4$	H; C[i]	*e*	*e*	*e*
11d acid	H	$(CH_2)_7CO_2H$	E	77*	$C_{24}H_{40}O_4$	*j*	*e*	*e*	*e*
10e	H	$(CH_2)_8CO_2C_2H_5$	N	14 (4)	$C_{27}H_{46}O_4$	C, H	4.38×10^{-3}	6.21×10^{-3}	*e*
11e	H	$(CH_2)_8CO_2C_2H_5$	E	22 (4)	$C_{27}H_{46}O_4$	C, H	*e*	*e*	*e*
10e acid	H	$(CH_2)_8CO_2H$	N	81*	$C_{25}H_{42}O_4$	C, H	*e*	*e*	*e*
11e acid	H	$(CH_2)_8CO_2H$	E	55*	$C_{25}H_{42}O_4$	C, H	*e*	*e*	*e*
10f	H	$(CH_2)_4C(CH_3)_2CH_2CO_2C_2H_5$	N	9 (5)	$C_{27}H_{46}O_4$	C, H	*e*	*e*	*e*
11f	H	$(CH_2)_4C(CH_3)_2CH_2CO_2C_2H_5$	E	18 (5)	$C_{27}H_{46}O_4$	C, H	*e*	*e*	*e*
10f acid	H	$(CH_2)_4C(CH_3)_2CH_2CO_2H$	N	83*	$C_{25}H_{42}O_4$	C, H	*e*	*e*	*e*
11f acid	H	$(CH_2)_4C(CH_3)_2CH_2CO_2H$	E	81*	$C_{25}H_{42}O_4$	C, H	*e*	*e*	*e*
10g	H	$(CH_2)_4SCH_2CO_2C_2H_5$	N	11 (15)	$C_{24}H_{40}O_4S$	C, H, S	300×10^{-6}	30×10^{-6}	909×10^{-6}
11g	H	$(CH_2)_4SCH_2CO_2C_2H_5$	E	14 (15)	$C_{24}H_{40}O_4S$	C, H; S[k]	*e*	$>672 \times 10^{-6}$	*e*
10g acid	H	$(CH_2)_4SCH_2CO_2H$	N	56*	$C_{22}H_{36}O_4S$	*l*	171×10^{-6}	91.2×10^{-6}	*e*
11g acid	H	$(CH_2)_4SCH_2CO_2H$	E	71*	$C_{22}H_{36}O_4S$	*m*	*e*	606×10^{-6}	*e*
14	HO	$(CH_2)_6CO_2H$	N	(23)	$C_{23}H_{38}O_5$	C, H	1.26×10^{-6}	555×10^{-9}	1.45×10^{-6}
15	HO	$(CH_2)_6CO_2H$	E		$C_{23}H_{38}O_5$	C, H	43.9×10^{-6}	17×10^{-6}	30×10^{-6}
16	HO	$CH_2CH{=}CH(CH_2)_3CO_2H$	N	12	$C_{23}H_{36}O_5$	C, H	2.51×10^{-6}	1.54×10^{-6}	1.06×10^{-6}
17	HO	$CH_2CH{=}CH(CH_2)_3CO_2H$	E	10	$C_{23}H_{36}O_5$	*n*	305×10^{-6}	48.4×10^{-6}	32.0×10^{-6}
18	HO	$CH_2CH{=}CH(CH_2)_3CO_2H$ (20-nor)	N	5	$C_{22}H_{34}O_5$	*o*	3×10^{-6}	97.9×10^{-9}	3×10^{-6}
19	HO	$CH_2CH{=}CH(CH_2)_3CO_2H$ (20-nor)	E	5	$C_{22}H_{34}O_5$	*p*	265×10^{-6}	46.7×10^{-6}	*e*

[a] Natural (N) and epi (E) configurations are assigned on the basis of relative TLC mobilities as compared to *l*-PGE_1 and its 15-epimer. See also ref 6. [b] Yield in parentheses refers to the yield of epimeric racemates. Yields designated with an asterisk are saponification yields. [c] Analyses indicated by letter only were within ±0.4% of the calculated values. [d] See Experimental Section and ref 21. [e] At 3.2 mg/kg the inhibition of bronchoconstriction was less than 50%. [f] Calcd for $C_{24}H_{40}O_4$: 392.2925. Found: 392.2934. [g] Calcd for $C_{24}H_{40}O_4$: C, 73.43. Found: C, 72.67. [h] Calcd for $C_{22}H_{36}O_4$: 364.2613. Found: 364.2609. [i] Calcd for $C_{24}H_{40}O_4$: C, 73.43. Found: C, 72.59. [j] Calcd for $C_{24}H_{40}O_4$: 392.2925. Found: 392.2930. [k] Calcd for $C_{24}H_{40}O_4S$: S, 7.55. Found: S, 8.01. [l] Product identified by TLC, IR, and NMR. [m] Calcd for $C_{22}H_{36}O_4S$: 396.2334. Found: 396.2324. [n] Calcd for $C_{23}H_{34}O_4$ (M - H_2O): 374.2457. Found: 374.2444. [o] Calcd for $C_{22}H_{32}O$ (M - H_2O): 360.2300. Found: 360.2302. [p] Calcd for $C_{22}H_{32}O_4$ (M - H_2O): 360.2300. Found: 360.2297.

at 0 °C for 15 min, the solution was stirred at ambient temperature for 18 h and then poured into 200 mL of hexanes. The solution was washed twice each with ice water and brine, dried, and evaporated. Distillation furnished 6.02 g (80%) of **6** as a colorless oil: bp 110–112 °C (14 mm); IR 3390, 2985, 2125, 870, and 844 cm^{-1}; NMR δ 4.24 [d, 1 H, HC≡CC*H*(OSiMe$_3$)–, J = 2 Hz], 2.34 [d, 1 H, *H*C≡CCH(OSiMe$_3$)–, J = 2 Hz], 0.95 (t, 3 H, –CH_2CH_3, J = 7 Hz), and 0.20 ppm [s, 9 H, Si(CH_3)$_3$]. Anal. ($C_{11}H_{26}OSi$) C, H.

(*E*)-1-Iodo-4,4-trimethylene-3-trimethylsilyloxy-1-octene (7). To a mixture of 4.76 g (0.126 mol) of $NaBH_4$ and 23.6 g (0.337 mol) of 2-methyl-2-butene in 220 mL of dry THF at –5 °C was added dropwise 23.8 g (0.168 mol) of freshly distilled boron trifluoride etherate. The resulting mixture was stirred at –5 to 0 °C for 2 h and to it was added dropwise a solution of 20 g (0.084 mol) of 4,4-trimethylene-3-trimethylsilyloxy-1-octyne (**6**) in 20 mL of dry THF, and stirring was continued at ambient temperature for 2.5 h. The mixture was cooled to –5 °C and 44 g (0.586 mol) of trimethylamine oxide was added portionwise over a period of 20 min, maintaining the temperature at 15–20 °C. The mixture was stirred at ambient temperature for 2 h and then poured simultaneously with a solution of 119 g (0.938 mol) of iodine in 290 mL of THF into 1490 mL of 15% aqueous NaOH solution. After stirring for 30 min, the organic phases were washed with 5% aqueous sodium thiosulfate solution and brine, then dried, and taken to dryness to give 27 g of residual oil. Chromatography on 135 g of Florisil and eluting with 500 mL of hexanes furnished 24 g of an oil which was shown to be contaminated with starting material and iodoform by IR and TLC. The material was further purified via the free alcohol in the following manner. The trimethylsilyl group was hydrolyzed by dissolving the above oil in 350 mL of acetic acid–THF–water (4:2:1) and stirring at ambient temperature for 5 min. The solvent then was removed under reduced pressure, and the residual oil containing mainly (*E*)-1-iodo-4,4-trimethylene-1-octen-3-ol was applied to a dry column (2 in. flat) containing 1200 g of Woelm silica gel. The column was developed with benzene, cut into 1-in. segments, and each segment was eluted with $CHCl_3$. Combination of the appropriate fractions afforded 300 mg of iodoform, 2.8 g of 4,4-trimethyl-1-octyn-3-ol, and 11.6 g of (*E*)-1-iodo-4,4-trimethylene-1-octen-3-ol. Silylation of this material in the manner described above followed by distillation of the residual oil furnished 13 g of **7**: bp 83–84 °C (0.2 mm); IR 2860, 1600, 880, and 840 cm^{-1}; NMR δ 6.60 (dd, 1 H, C-2 H, $J_{2,3}$ = 6 Hz, $J_{1,2}$ = 14 Hz), 6.22 (d, 1 H, C-1 H, $J_{1,2}$ = 14 Hz), 3.98 (d, 1 H, C-3 H, $J_{2,3}$ = 6 Hz), 0.93 (t, 3 H, C-3 H), and 0.10 ppm [s, 9 H, Si(CH_3)$_3$]. Anal. ($C_{14}H_{27}IOSi$) C, H, I.

2-[(*Z*)-6′-Carbethoxy-2′-hexenyl]cyclopent-2-en-1-one (9b).[16] A solution of 23.9 g of 2-[(*Z*)-6′-carboxy-2′-hexenyl]-cyclopent-3-en-1-ol[17] in 800 mL of acetone was cooled to 0 °C and treated dropwise with 63 mL of standard Jones reagent. After 15 min, the reaction was quenched with 2-propanol and the acetone was removed under reduced pressure. The residue was taken up in water, saturated with NaCl, and extracted several times with ethyl acetate. The combined extracts were washed with saturated NaCl solution, dried, and taken to dryness to give 18.3 g of 2-[(*Z*)-6′-carboxy-2′-hexenyl]cyclopent-3-en-1-one.

Without further purification the cyclopent-3-en-1-one was isomerized to the corresponding cyclopent-2-en-1-one in the following manner. A solution of this cyclopent-3-en-1-one (43 g) in 1 L of absolute MeOH containing 23 mL (1.15 equiv) of piperidine was kept at ambient temperature for 18 h and then taken to dryness. The residual oil was partitioned between ethyl acetate and water, and the resultant mixture, stirred in an ice bath, was acidified with dilute HCl. The separated aqueous phase was extracted several times with ethyl acetate. The combined extracts were washed with saturated NaCl solution, dried, and taken to dryness to give 39.7 g of crude 2-[(*Z*)-6′-carboxy-2′-hexenyl]-cyclopent-2-en-1-one as an oil; λ_{max} 223 nm (ϵ 7300) indicated the product to be about 80% real; TLC (60:40:2 cyclohexane–ethyl acetate–acetic acid) major spot R_f 0.4.

A solution of the above crude acid (40 g) in 1500 mL of absolute alcohol containing 2.7 g of *p*-toluenesulfonic acid was stirred at reflux temperature under argon atmosphere for 18 h and then taken to dryness. A solution of the residual oil in ether was washed with saturated $NaHCO_3$ solution and saturated NaCl solution, dried, and taken to dryness to give 42 g of oil. Distillation furnished 30.2 g of 2-[(*Z*)-6′-carbethoxy-2′-hexenyl]cyclopent-2-en-1-one (**9b**): bp 143 °C (0.2 mm); IR 1739, 1700, and 1637 cm^{-1}; λ_{max}^{EtOH} 225 nm (ϵ 900); NMR δ 7.40 (m, 1 H, C=CH–), 5.55 (m, 2 H, –HC=CH–), 4.15 (q, 2 H, –OCH_2), 1.25 (t, 3 H, –CH_3). Anal. ($C_{14}H_{20}O_3$) H; C: calcd, 71.16; found, 70.66.

General Conjugate Addition Procedure. Preparation of *dl*-11-Deoxy-16,16-trimethyleneprostaglandin E$_1$ Ethyl Ester (10a) and 15-Epimer 11a. To a stirred solution of 5 g (13.7 mmol) of (*E*)-1-iodo-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**7**) in 5 mL of dry ether cooled to –78 °C in an argon atmosphere was added 40 mL (30.0 mmol) of *t*-BuLi in pentane. After stirring for 35 min, the solution was allowed to warm to –5 °C and stirred for an additional 1 h. It was then cooled to –78 °C and there was added a solution containing 1.79 g (13.7 mmol) of copper(I) pentyne (CuC≡CC_3H_7)[13] in 5.5 mL of hexamethylphosphorous triamide and 50 mL of dry ether. The resulting solution containing lithiopentynyl [(*E*)-4,4-trimethylene-3-trimethylsilyloxy-1-octenyl]cuprate (**8**) was stirred at –78 °C for 1 h at which point 3.26 g (13.7 mmol) of 2-(6-carbethoxyhexyl)cyclopent-2-en-1-one (**9a**)[15] in 30 mL of ether was added. The solution was stirred at –15 °C for 1 h and then at 0 °C for 1 h and then poured into 600 mL of saturated NH_4Cl solution and 200 mL of ether and stirred for 20 min. The ether layer was separated and the aqueous layer was extracted twice with ether. The combined ether extracts were washed with water and dried. After solvent evaporation, the residual oil was dissolved in a small amount of ice-cold hexanes, filtered from solids, and taken to dryness to furnish 8.0 g of an oil. The trimethylsilyl group was removed by treating the oil with 125 mL of acetic acid–THF–water (4:2:1) at ambient temperature for 10 min followed by solvent evaporation to afford 5.63 g of oil. The oil was applied to a dry column (2 in. flat) using 950 g of silica gel and developed with ethyl acetate–benzene (1:4). Eluent (150 mL) was collected and discarded. The column was divided into 1-in. segments and segments 27–32 were combined to give 1.37 g (25%) of **10a** as a yellow oil: IR 3570, 1740, and 975 cm^{-1}; NMR δ 5.70 (m, 2 H, C-13,14 H), 4.20 (q, 2 H, OCH_2CH_3, J = 7 Hz), 4.10 (m, 1 H, C-15 H), and 0.97 ppm (t, 3 H, C-20 H). Anal. ($C_{25}H_{42}O_4$) C, H.

Segments 16–25 were combined to give 2.16 g (38%) of **11a** as a yellow oil: IR 3570, 1740, and 975 cm^{-1}; NMR δ 5.70 (m, 2 H, C-13,14 H), 4.20 (q, 2 H, OCH_2CH_3, J = 7 Hz), 4.10 (m, 1 H, C-15 H), and 0.97 ppm (t, 3 H, C-20 H). Anal. ($C_{25}H_{42}O_4$) C, H.

This method was employed for the preparation of the *dl*-11-deoxy-16,16-trimethylene esters **10b–g** and their epimers (see Table I).

General Saponification Procedure. Preparation of *dl*-11-Deoxy-16,16-trimethyleneprostaglandin E$_1$ (10a Acid). A mixture of 500 mg (1.2 mmol) of *dl*-11-deoxy-16,16-trimethyleneprostaglandin E$_1$ ethyl ester (**10a**) and 20 mL of a solution of MeOH–water (1:1) containing 336 mg of KOH was refluxed for 2 h. The reaction mixture was cooled and extracted with ether. The aqueous phase was acidified with 5% HCl and extracted with ether. The ether extracts were washed with brine, dried, and concentrated in vacuo to provide 421 mg (92%) of **10a** acid as a yellow oil: R_f 0.36 [cyclohexane–EtOAc–acetic acid (60:40:2)]; NMR 6.45 (br s, 2 H, OH), 5.68 (m, 2 H, C-13,14 H), 4.03 (m, 1 H, C-15 H), and 0.98 ppm (t, 3 H, C-20 H). Anal. ($C_{23}H_{38}O_4$) C, H.

The various *dl*-11-deoxyprostaglandin acids listed in Table I were prepared by this procedure.

***dl*-16,16-Trimethyleneprostaglandin E$_1$ (14) and 15-Epimer 15.** In the manner described below for the preparation of *dl*-16,16-trimethyleneprostaglandin E$_2$ (**16**), treatment of 6.59 g (17.74 mmol) of **7** in dry ether with 44.35 mL (35.48 mmol) of *t*-BuLi, followed by 2.32 g (17.74 mmol) of copper(I) pentyne,[13] furnished the lithiopentynyl cuprate **8**, which in turn was treated with 7.09 g (17.74 mmol) of 4-tetrahydropyranyloxy-2-(6-carbotetrahydropyranyloxyhexyl)cyclopent-2-en-1-one (**12**)[19] to afford 10.97 g of oil. This oil was dissolved in 300 mL of acetic acid–THF–water (4:3:1). The mixture was stirred at ambient temperature for 4 h, poured into 200 mL of iced water, and extracted with ether. The ether extracts were taken to dryness to furnish 8.39 g of an oil. This material was applied to a dry column (2 in. flat) using 1000 g of silica gel and developed with 30% EtOAc in hexanes containing 0.5% acetic acid. Eluent (700 mL) was collected and

was discarded. The column was cut into 1-in. segments and segments 35-39 (from bottom) were combined to provide 1.97 g (23%) of 11-*O*-tetrahydropyranyl-16,16-trimethyleneprostaglandin F$_{2\alpha}$: IR 3500–2600 (br), 1739, 1712, and 975 cm^{-1}; NMR δ 6.10 (s, 2 H, OH), 5.75 (m, 2 H, C-13,14 H), 4.05 (m, 2 H, C-11,15 H), and 0.98 ppm (t, 3 H, C-20 H).

The above oil was treated with 140 mL of acetic acid–THF–water (4:2:1) at 45 °C for 4.5 h, and the resulting solution was taken to dryness to furnish 1.54 g of oily material, which was applied to a dry column (1.5 in. flat) using 450 g of silica gel and developed with cyclohexane–EtOAc–acetic acid (60:40:2). After elution of 650 mL of solvent and removal of 12 in. from the bottom, the remainder of the column was divided into 1-in. segments. Segments 15–19 were combined to furnish 312 mg of **15**: R_f 0.38 [cyclohexane–EtOAc–acetic acid (60:40:2), three elutions]; NMR δ 6.17 (m, 2 H, OH), 5.68 (m, 2 H, C-13,14 H), 4.05 (m, 2 H, C-11,15 H) and 0.88 ppm (t, 3, C-20 H). Anal. ($C_{23}H_{38}O_5$) C, H. Segments 22–29 were combined to provide 249 mg of **14**: R_f 0.29 [cyclohexane–EtOAc–acetic acid (60:40:2), three elutions]: NMR δ 6.18 (m, 2 H, OH), 5.70 (m, 2 H, C-13,14 H), 4.10 (m, 2 H, C-11,15 H), and 0.90 ppm (t, 3 H, C-20 H). Anal. ($C_{23}H_{38}O_5$) C, H.

***dl*-16,16-Trimethyleneprostaglandin E$_2$ (16) and 15-Epimer 17.** To a solution of 7.9 g (21.6 mmol) of vinyl iodide 7 in 16 mL of hexanes, cooled to −78 °C under an argon atmosphere, was added 60 mL (47 mmol) of *t*-BuLi. After stirring for 45 min, the solution was allowed to warm to −5 °C and stirred for an additional hour. This solution was cooled to −78 °C, and there was added a solution of 2.83 g (21.6 mmol) of copper(I) pentyne[13] in 8.7 mL of hexamethylphosphorous triamide and 50 mL of dry ether. The solution was stirred at −78 °C for 1 h. To this solution, containing the lithiopentynyl cuprate **8**, was added a solution of 8.45 g (21.5 mmol) of 4-tetrahydropyranyloxy-2-[(*Z*)-6-carbotetrahydropyranyloxy-2-hexenyl]cyclopent-2-en-1-one (**13**)[20] in 50 mL of dry ether. The solution was stirred at −15 °C for 1 h and at 0 °C for 1 h and then poured into 1 L of saturated NH_4Cl solution and 300 mL of ether and stirred for 20 min. The ether phase was separated and the aqueous phase was extracted twice with ether. The combined ether extracts were treated with 5% sodium thiosulfate solution and brine, dried, and taken to dryness to furnish 16 g of an oil.

This oil was dissolved in 400 mL of acetic acid–THF–water (20:10:3) and the mixture was heated at 40 °C for 4 h with stirring. The solids were removed by filtration and the filtrate was taken to dryness to furnish an oil. This crude product was applied to a dry column (2 in. flat) using 1500 g of silica gel and developed with cyclohexane–EtOAc–acetic acid (60:4:2). Eluent (400 mL) was collected and discarded. Twelve inches were removed from the bottom of the column, and the remainder of the column was divided into 1-in. segments. Segments 52–57 were combined to furnish 841 mg (10%) of **17**: R_f 0.15 [cyclohexane–EtOAc–acetic acid (60:40:2)]; IR 3500–2666 (br), 1745, 1709, and 973 cm^{-1}; NMR δ 6.12 (br s, 3 H, O*H*), 5.68 (m, 2 H, C-13,14 H), 5.38 (m, 2 H, C-5,6 H), 4.05 (m, 2 H, C-11,15 H), and 0.95 ppm (t, 3 H, C-20 H). Anal. Calcd for $C_{23}H_{34}O_4$ (M − H_2O): mol wt 374.2457. Found: mol wt 374.2444.

Segments 60–66 were combined to furnish 1.059 g (12%) of **16**: R_f 0.124 [cyclohexane–EtOAc–acetic acid (60:40:2)]; IR 3571–2631 (br), 1740, 1709, and 973 cm^{-1}; NMR δ 6.12 (br s, 2 H, OH), 5.68 (m, 2 H, C-13,14 H), 5.37 (m, 2 H, C-5,6 H), 4.05 (m, 2 H, C-11,15 H), and 0.87 ppm (t, 3 H, C-20 H). Anal. ($C_{23}H_{36}O_5$) C, H.

Guinea Pig Bronchodilator Assay.[21] Hartley guinea pigs of either sex, weighing 250–500 g, were anesthetized by intraperitoneal injection of urethane (1.5 g/kg) and given an intravenous injection of gallamine. They were artificially ventilated through a tracheal cannula (60 strokes/min), the respiratory volume being adjusted according to the weight of the animal and the rate of the pump.[22] The two jugular veins were catheterized, the first one being used for curarization and administration of the drugs, the second for the injection of the spasmogenic agent. This injection was made at different selected speeds with a perfusion pump (Braun Unita 1). The intratracheal pressure was measured with a transducer (Sanborn 267 AC) connected to the tracheal cannula and recorded on a Sanborn polygraph.

Bronchial spasms were produced by intravenous injection of acetylcholine, histamine, or serotonin. The speed of injection of the spasmogenic solution and its concentration were chosen to produce an increase of the tracheal pressure of 20–50 cm of water. For acetylcholine that dose varied from 40 to 150 μg/kg, for histamine from 5.6 to 22.5 g/kg, and for serotonin from 7.5 to 30 μg/kg.

Injections of 12-s duration were repeated every 5 min throughout the assay. When three successive control bronchoconstrictions of similar intensity were obtained, the animal was considered to be ready for the assays and received the first dose of the candidate drug 2 min later.

Water-soluble compounds (sodium salts) were injected through the jugular vein. The drug injection took 1 min and was repeated three or four times per animal at 15-min intervals so that three of four doses, in logarithmic progression, were assayed. Water-insoluble compounds (esters) were dispersed in 10% aqueous ethanol and administered in the same way.

The broncholytic activity of each compound was measured in at least four guinea pigs for each of the three spasmogenic substances. The amplitude of the spasms (i.e., the difference between the maximum total tracheal pressure during the spasms and the normal insufflation pressure without spasm) following the administration of the drug was expressed in cm of water. For each spasm the mean difference vs. the control [i.e., Σ (treated spasm − control spasm)/(control spasm)] was calculated. For an easier expression of the results, the mean difference was transformed into a percentage of variation. When the percent of variation of the first spasm following any dose of the drug reached at least −50%, a regression line of the percent variation vs. the dose was computed in a semilogarithmic system. The ED_{50} (dose producing a −50% variation) was then calculated. When a compound is not active enough to allow the calculation of an ED_{50}, the ED_{50} is considered to be greater than the maximal dose administered.

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Cholecystokinin (Pancreozymin). 3.[1] Synthesis and Properties of an Analogue of the C-Terminal Heptapeptide with Serine Sulfate Replacing Tyrosine Sulfate

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The influence of tyrosine *O*-sulfate, the 27th residue in the sequence of cholecystokinin (pancreozymin) (CCK-PZ) on calcium outflux in isolated pancreatic cells of guinea pigs, was studied by replacing this residue in the biologically active C-terminal heptapeptide, CCK-PZ-(27–33) (I), with L-serine *O*-sulfate. The synthetic analogue Ser-(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (IV), produced the half-maximal outflux observed with I, if applied at about 250 times higher concentration. The unsulfated form of IV was about ten times less potent than unsulfated I. Thus, in the effect on the calcium outflux, serine cannot replace tyrosine without a major loss in potency; a sulfate ester group in position 27 is important but in itself not sufficient for full potency. Interestingly, if the terminal amino group of the heptapeptide is left protected by a *tert*-butyloxycarbonyl group, the potencies of the derivatives of both I and IV were slightly, but significantly, higher than those of the free peptides.

A gastrointestinal hormone stimulating the contraction of the gall bladder was discovered by Ivy and Oldberg[2] and was named cholecystokinin (CCK), while a factor causing the release of digestive enzymes from the pancreas was found by Harper and Raper[3] in the intestinal mucosa of the hog and was designated as pancreozymin (PZ). Subsequently, Jorpes and Mutt[4] demonstrated that a single compound is responsible for both kinds of activities. The sequence of the 33 amino acid residues in the peptide chain of CCK-PZ was elucidated by Mutt and Jorpes.[5] The C-terminal dodecapeptide of CCK-PZ, synthesized by Ondetti and his co-workers,[6] and even the C-terminal heptapeptide, Tyr(*O*-SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2 (I), showed the hormonal properties of the entire molecule. Similar biological activities are exhibited by caerulein, a peptide isolated from the skin of *Hyla caerulea* by Anastasi and her associates.[7] The C-terminal sequences of CCK-PZ and caerulein are quite similar, except that methionine in position 28 of CCK-PZ is replaced by threonine in caerulein. Among the synthetic peptides related to caerulein, the heptapeptide with the C-terminal sequence of CCK-PZ was also investigated.[8] A closely related sequence is found also in the antral hormone gastrin.[9]

Unlike in gastrin, where the presence or absence of a sulfate ester group on the phenolic hydroxyl of tyrosine has no major influence on biological activity, in CCK-PZ the fact that a tyrosine *O*-sulfate residue and not a simple tyrosine is present is quite significant. The hormonally active C-terminal peptides tested before sulfation had only a small fraction of the potency of the corresponding sulfated materials.[6,8,10] Also, the recently reported[11] synthesis of Tyr^{27}-CCK-PZ produced a peptide which was 250 times less active than the parent hormone. Yet, while these observations provided ample evidence on the importance of the sulfate ester grouping, it was still questionable whether or not a sulfate ester in the side chain of the 27th residue is *sufficient* for full hormonal activity. The role of the structural features of *tyrosine* remained to be investigated. We decided, therefore, to synthesize the shortest part of the CCK-PZ molecule with significant biologic activity, the C-terminal heptapeptide CCK-PZ-(27–33), with serine *O*-sulfate instead of tyrosine *O*-sulfate in position 27. The potency, if any, of this analogue was expected to shed light on the role of the tyrosine moiety.

In previous studies CCK-PZ as well as synthetic C-terminal fragments have been shown to increase amylase secretion from dispersed acinar cells from the guinea pig pancreas.[10] The initial steps in the action of CCK-PZ on enzyme secretion are release of exchangeable cellular calcium followed by an increase in cellular 3′,5′-guanosine monophosphate.[12,13] Previous results[10,12,13] indicate that stimulation of calcium outflux from pancreatic acinar cells is a sensitive, reproducible bioassay system for CCK-PZ and structurally related peptides.

Synthesis. The protected heptapeptide amide *tert*-butyloxycarbonyl-L-seryl-L-methionylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine amide (II) was synthesized stepwise,[14] starting with the C-terminal