11,12-Secoprostaglandins. 2. *N*-Acyl-*N*-alkyl-7-aminoheptanoic Acids

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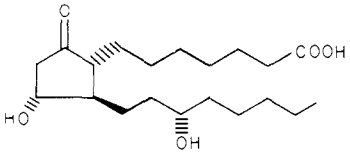
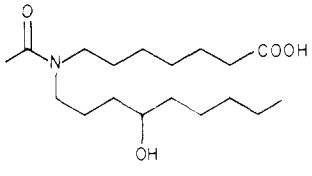
A series of *N*-acyl-*N*-alkyl-7-aminoheptanoic acids has been prepared and evaluated for their ability to mimic the natural prostaglandins in certain biological systems. These compounds can be regarded as 8-aza-11,12-secoprostaglandins and, indeed, most of them stimulate cAMP formation in the mouse ovary assay, just as is observed with the natural prostaglandins. Selected compounds from this series also have been studied and shown to have prostaglandin-like effects in vivo.

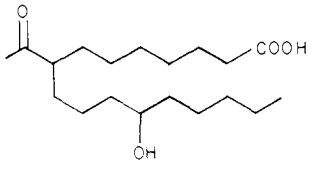
We recently described the synthesis and biological evaluation of a group of 11,12-secoprostaglandin analogues (acylhydroxyalkanoic acids).[1] The prostaglandin-like activities in vitro and in vivo of certain of these compounds (e.g., **12**) prompted the preparation and evaluation of a series of 8-aza analogues[2,3] (e.g., **5**) with which this paper is concerned. The substitution of a nitrogen atom for the carbon atom in position 8 of the 11,12-secoprostaglandins reduces the number of stereoisomers, and the planar amide function so produced introduces an element of rigidity into these molecules.



13,14-dihydro-PGE$_1$



5



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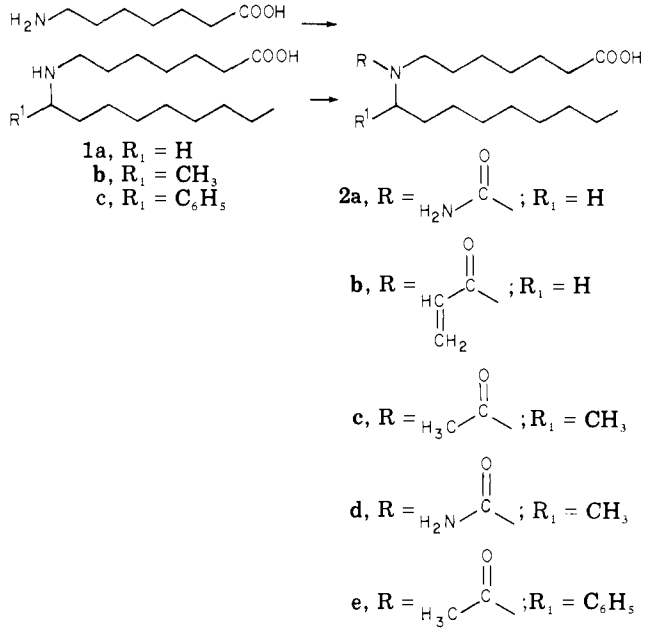
Chemistry. The carboxylic acids represented by **2** in Scheme I were prepared by reaction of the corresponding substituted aminoheptanoic acids with acetic anhydride (**2c,e**), potassium cyanate (**2a**), urea (**2d**), or acryloyl chloride (**2b**) (see Table I). The substituted aminoheptanoic acids (**1**) resulted from the reductive alkylation of 7-aminoheptanoic acid with the corresponding aldehyde or ketone using the Borch[4] procedure. The product acids (**5**, **9**, and **10**) in Scheme II were prepared by saponification of the corresponding acetoxy esters. For the acids **9a–c**, where the hydrolysis of the cyanamido group of **8a–c** to the ureido group also was required, this hydrolysis was accomplished using alkaline hydrogen peroxide. Compound **11** was prepared by light-catalyzed addition of thiolacetic acid to the cyanamide **10** followed by basic hydrolysis of the adduct. The acetoxy esters **4** and **8** were formed by alkylation of the corresponding amide (**3**) or cyanamide (**7**) with the appropriate acetoxyalkyl halide. Treatment of 7-aminoheptanoic acid with acetic anhydride followed by esterification afforded ester **3**. Treatment of

Scheme I



1a, R_1 = H
b, R_1 = CH_3
c, R_1 = C_6H_5

2a, R = H_2NCOCH_2; R_1 = H

b, R = $H_2C{=}C(H)COCH_2$; R_1 = H

c, R = H_3CCOCH_2; R_1 = CH_3

d, R = H_2NCOCH_2; R_1 = CH_3

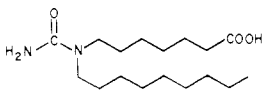
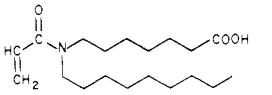
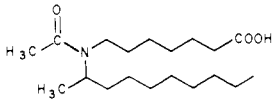
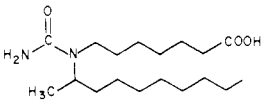
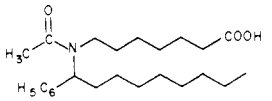
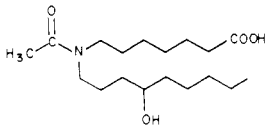
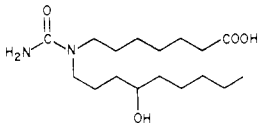
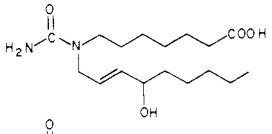
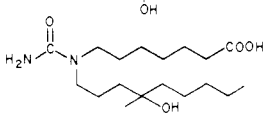
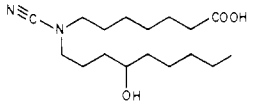
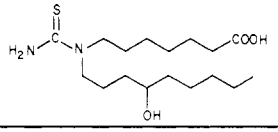
e, R = H_3CCOCH_2; R_1 = C_6H_5

7-aminoheptanoic acid with potassium cyanate followed by esterification provided ester **6** which was dehydrated to the cyanamide **7** by the Sheehan procedure.[5]

Biological Activity. Prostaglandins of the *E* series have been shown to raise cAMP levels in cells of many types.[6] This dose-related stimulation by PGE_1 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.[7] In this assay, described in detail in the Experimental Section, the cAMP-^{14}C formed is isolated and measured. Results are expressed as fold increases in cAMP formation obtained by dividing the cAMP levels in treated ovaries by those levels found in untreated ovaries.

In Table II, two of the 8-aza-11,12-secoprostaglandins (**5** and **9a**) are compared with PGE_1 and tetrahydro-PGA_1 for their ability to stimulate the formation of cAMP in mouse ovaries. Also included is 8-acetyl-12-hydroxyheptadecanoic acid (**12**), a representative compound from the first paper[1] of this series. Compound **5** which is the 8-aza isostere of 8-acetyl-12-hydroxyheptadecanoic compares favorably with it in its ability to increase cAMP levels in this assay. The corresponding urea, compound **9a**, is equally active at the low concentration and shows a somewhat greater activity at the higher concentrations, thus confirming our original speculation. Further variations of the structure of **9a** to include the 13,14 double bond (PG numbering) (compound **9b**) or a 15-methyl group (**9c**) produced a slight decrease in activity (see Table

Table I

No.		Yield, %	R_f[a]	Formula[b]	Mouse ovary assay, fold increase in cAMP 10[f]	25[f]	100[f]
2a[c]		30	0.52	$C_{17}H_{34}N_2O_3$	15	16	25
2b		26	0.7	$C_{19}H_{35}NO_3 \cdot 0.5H_2O$	2	7	11
2c		92	0.69	$C_{19}H_{37}NO_3$		3	4
2d[d]		28	0.58	$C_{18}H_{36}N_2O_3$	9	16	17
2e		91	0.69	$C_{24}H_{39}NO_3$		1	1
5		43	0.55	$C_{18}H_{35}NO_4$	10	17	17
9a[e]		87	0.41	$C_{17}H_{34}N_2O_4$	23	43	40
9b		38	0.31	$C_{17}H_{32}N_2O_4 \cdot H_2O$	14	19	26
9c		19	0.35	$C_{18}H_{36}N_2O_4$	3	8	22
10		60	0.60	$C_{17}H_{32}N_2O_3 \cdot 0.5H_2O$	4	18	31
11		71	0.50	$C_{17}H_{34}N_2O_3S$	4	16	26

[a] Determined on SiO_2 plates with $CHCl_3$-CH_3OH-AcOH (89:10:1). [b] All compounds were analyzed for C, H, and N. Analytical results were within 0.4% of the theoretical values except as noted for 2e in the Experimental Section. [c] Mp 73-74 °C, crystallized from hexane. [d] Mp 95-96 °C, crystallized from hexane. [e] Mp 75-76 °C, crystallized from ether. [f] Concentration in μg/ml.

I). The cyanamido compound **10** and the thiourea **11** were very active at the higher concentration, but the activity rapidly decreased as the drug concentration was lowered. The observation that removal of the hydroxy group from **9a** gave us a compound (**2a**) that retained good activity prompted further structural variations using this simpler model. Compound **2d**, which contains a methyl group in a position that corresponds to the 12 position of PGE_1, retained the activity of its parent (**2a**). However, substitution of a phenyl group in that position, to give **2e**, completely destroyed activity.

The evaluation of these 8-aza-11,12-seco analogues in vivo is in progress in these laboratories, and these results will be published elsewhere. It can be stated that certain of these compounds do show the characteristic actions of the prostaglandins in animals. For example, **9a**, in aqueous solution as the sodium salt, infused into the thoracic aorta of an anesthetized 25-kg dog at a rate of 0.25 mg/kg/min, increased renal blood flow from 188 to 258 ml/min. The renal blood flow was measured in one kidney by electromagnetic blood flow probes and reached the maximum 10 min after the beginning of the infusion.

Experimental Section

Melting points were taken in open capillary tubes and are uncorrected as are boiling points. ^1H NMR spectra were obtained

Scheme II

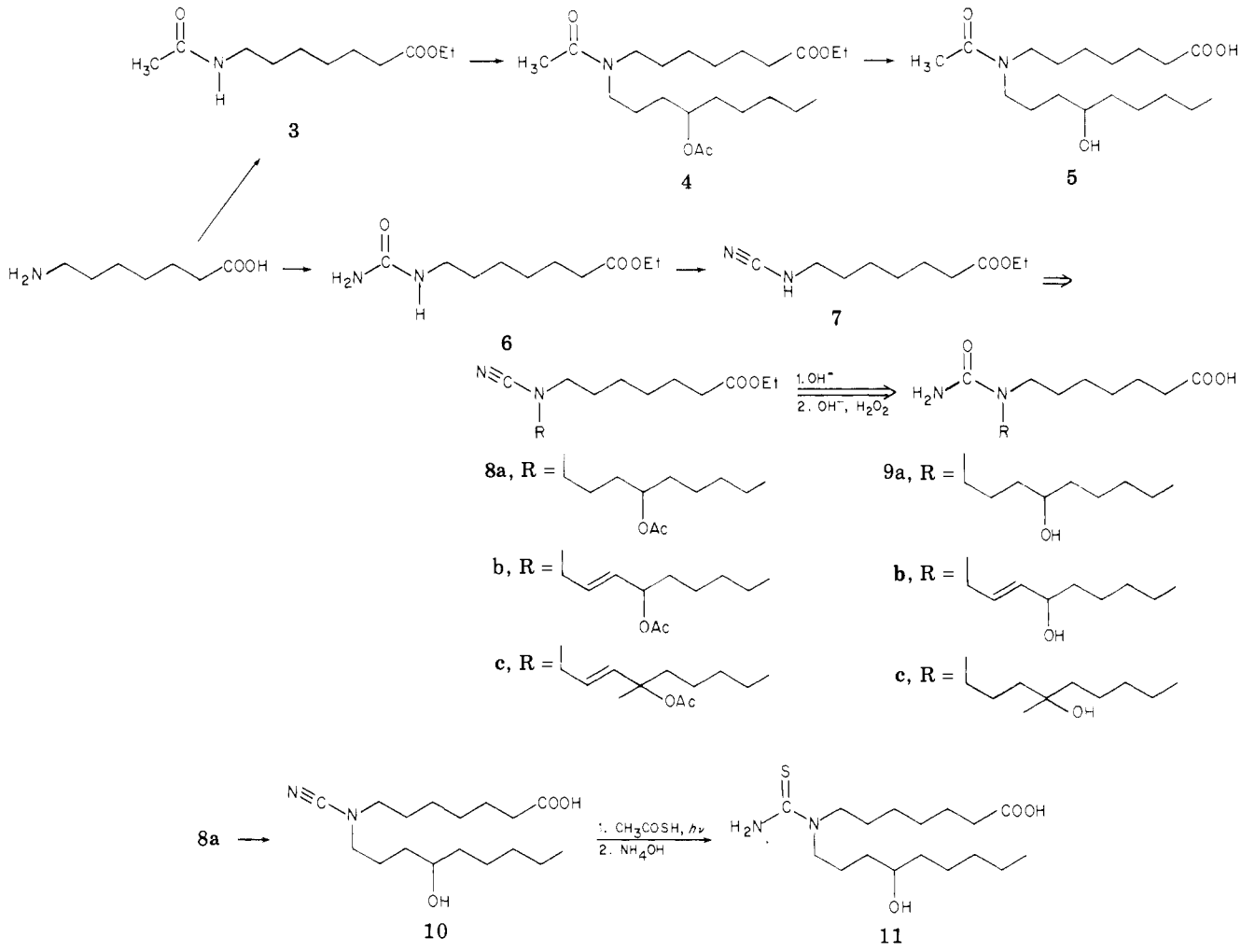
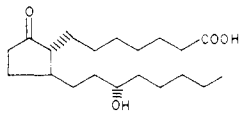
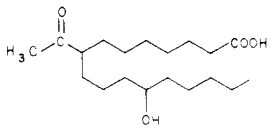


Table II

	Mouse ovary PG assay, fold increase in cAMP					
	0.05[a]	0.1[a]	1.0[a]	10[a]	25[a]	100[a]
PGE_1	29	25	54			
			10	25	26	
 12			2	11	14	23
5				10	17	17
9a			2	23	43	40

[a] Concentrations in μg/ml.

in $CDCl_3$ on a Varian A-60A spectrometer and chemical shifts are reported as parts per million relative to Me_4Si as an internal standard.

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.

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.

7-(1-Nonylureido)heptanoic Acid (2a). (a) 7-Nonylaminoheptanoic Acid Hydrobromide (1a). A methanol (25 ml) solution of nonanal (1.42 g, 10 mmol), 7-aminoheptanoic acid (1.45 g, 10 mmol), and $NaCNBH_3$ (943 mg, 15 mmol) was stirred at room temperature for 2 h. The reaction mixture was added to water (75 ml) and then made definitely acidic with 48% HBr. A solid separated which was recovered by filtration. This solid was recrystallized from EtOH–H_2O containing a few drops of 48% HBr to yield 600 mg (18%) of **1a**, mp 151–152 °C. Anal. ($C_{16}H_{33}NO_2$·HBr) C, H, N.

(b) 7-(1-Nonylureido)heptanoic Acid (2a). A water (10 ml) solution of **1a** (1.5 g, 4.2 mmol) and KCNO (342 mg, 4.2 mmol) acidified to congo red with dilute HCl was heated on the steam bath for 2 h. The reaction was cooled and an oil separated which was extracted into ethyl acetate. The organic phase was dried

and then evaporated to yield an oil that crystallized when triturated with hexane. There was obtained 400 mg (30%) of **2a**, mp 73–74 °C. Anal. ($C_{17}H_{34}N_2O_3$) C, H, N.

7-(*N*-Nonylacrylamido)heptanoic Acid (2b). To a solution of **1a** (1.0 g, 2.8 mmol) in MeOH (20 ml) was added KOH (313 mg, 5.6 mmol) in MeOH (12 ml). After 20 min the solvent was removed in vacuo and replaced with ether (30 ml). To the cooled, stirred suspension was added acryloyl chloride (560 mg, 6.2 mmol) and stirring was continued at room temperature for 20 h. The reaction mixture was poured into ice water (100 ml), carefully acidified with dilute HCl, and extracted with ether (3 × 75 ml). The organic phase was dried with Na_2SO_4 and then concentrated to an oil. This oil was chromatographed over silica gel with 3% MeOH in $CHCl_3$ as eluent. There was obtained 250 mg (26%) of **2b** as a viscous oil. Anal. ($C_{19}H_{35}NO_3 \cdot 0.5H_2O$) C, H, N.

7-[*N*-(1-Methylnonyl)acetamido]heptanoic Acid (2c). (a) 7-(1-Methylnonylamino)heptanoic Acid Hydrochloride (1b). Using the procedure used for the preparation of **1a** except that concentrated HCl was used in place of 48% HBr, 2-decanone was converted to **1b** in 28% yield. The product was purified by crystallization from ethyl acetate: mp 115–116 °C. Anal. ($C_{17}H_{35}NO_2 \cdot HCl$) C, H, N.

(b) 7-[*N*-(1-Methylnonyl)acetamido]heptanoic Acid (2c). A solution of **1b** (5.0 g, 13.6 mmol) in acetic anhydride (50 ml) was stirred and heated on the steam bath for 20 h. The reaction mixture was concentrated in vacuo; the remaining oil was dissolved in ether (100 ml), washed with brine, and then dried over Na_2SO_4. The solvent was removed in vacuo and the residue chromatographed on silica gel using 5% MeOH in $CHCl_3$ as eluent. There was obtained 4.1 g (92%) of **2c** as a viscous oil. Anal. ($C_{19}H_{37}NO_3$) C, H, N.

7-[*N*-(1-Methylnonyl)ureido]heptanoic Acid (2d). A water (60 ml) solution containing **1b** (6.0 g, 19 mmol), urea (4.8 g, 80 mmol), and 6 drops of 48% HBr was stirred and heated on the steam bath for 20 h. The cooled reaction mixture was acidified with dilute HCl and extracted with ethyl acetate and the organic phase dried over Na_2SO_4 and then concentrated in vacuo. The residual oil was chromatographed on silica gel using 10% MeOH in $CHCl_3$ as eluent. There was obtained 3.4 g (28%) of **2d**, mp 95–96 °C. The product was crystallized from hexane for analysis with no change in melting point. Anal. ($C_{18}H_{36}N_2O_3$) C, H, N.

7-[*N*-(1-Phenylnonyl)acetamido]heptanoic Acid (2e). (a) 7-(1-Phenylnonylamino)heptanoic Acid Hydrochloride (1c). Using the procedure used for the preparation of **1a**, except that concentrated HCl was used in place of 48% HBr, nonanophenone was converted to 1c in 10% yield. The product was purified by crystallization from CH_3CN: mp 143–145 °C. Anal. ($C_{22}H_{37}NO_2 \cdot HCl$) C, H, N.

(b) 7-[*N*-(1-Phenylnonyl)acetamido]heptanoic Acid (2e). The compound was prepared by the procedure described for **2c** from 1c (1.6 g, 4.2 mmol) and acetic anhydride (15 ml). There was obtained 1.5 g (91%) of **2e** as a viscous oil. Anal. ($C_{24}H_{39}NO_3$) H, N; C: calcd, 73.99; found, 73.52.

7-[*N*-(4-Hydroxynonyl)acetamido]heptanoic Acid (5). (a) Ethyl 7-Acetamidoheptanoate (3). A mixture of 7-aminoheptanoic acid (24 g, 165 mmol), acetic anhydride (40.8 g, 400 mmol), and water was stirred at room temperature for 3 h; then volatile materials were removed in vacuo. The liquid residue was dissolved in a solution made up of benzene (100 ml), ethanol (40 ml), and concentrated H_2SO_4 (1 ml) and heated at reflux for 24 h while the water produced was removed by a Dean-Stark apparatus. The cooled reaction mixture was poured into water (400 ml) and the pH adjusted to 10 by the addition of concentrated Na_2CO_3 solution. This mixture was extracted with ether; the organic layer was separated and dried over $MgSO_4$. Distillation afforded **3** (20.5 g, 57%), bp 131–136 °C (0.05 mm). Anal. ($C_{11}H_{21}NO_3$) C, H, N.

(b) Ethyl 7-[*N*-(4-Acetoxynonyl)acetamido]heptanoate (4). Ethyl 7-acetamidoheptanoate (4 g, 18.6 mmol) was added during 30 min to a stirred suspension of NaH (445 mg, 18.6 mmol) (50% in mineral oil) in benzene (20 ml) and DMF (20 ml). After 1 h of additional stirring 1-chloro-4-acetoxynonane[1] (4.1 g, 18.6 mmol) was added and the mixture was heated at 100 °C for 20 h. The cooled reaction mixture was poured into water (100 ml), cooled in an ice bath, and carefully acidified with dilute HCl. This mixture was extracted with benzene (2 × 75 ml). The benzene layer was washed with brine and dried over Na_2SO_4, and the solvent was removed in vacuo. The residue was purified by chromatography on silica gel with 3% MeOH in $CHCl_3$ as eluent. There was obtained 1.0 g (13%) of **4** as a viscous oil. Anal. ($C_{22}H_{41}NO_5$) C, H, N.

(c) 7-[*N*-(4-Hydroxynonyl)acetamido]heptanoic Acid (5). A stirred solution of ethanol (90 ml), water (10 ml), and NaOH (1.35 g, 33.8 mmol) was added to 4 (4.7 g, 11.8 mmol). The solution was stirred for an additional 20 h at room temperature and then diluted with water (150 ml). This cloudy solution was extracted once with ether (75 ml) and then carefully acidified with dilute HCl. The oil that separated was extracted into ether; the ether layer was dried over Na_2SO_4 and then evaporated in vacuo to give 2.0 g (43%) of **5** as a viscous oil: NMR δ 0.90 (3 H, m, CH_2CH_3), 2.20 (3 H, s, CH_3CO), 7.1 (2 H, m, COOH, OH). Anal. ($C_{18}H_{35}NO_4$) C, H, N.

7-[1-(4-Hydroxynonyl)ureido]heptanoic Acid (9a). (a) Ethyl 7-Ureidoheptanoate (6). A water (30 ml) solution containing 7-aminoheptanoic acid (2.9 g, 20 mmol) and KCNO (1.62 g, 20 mmol) was acidified to congo red with dilute HCl and then heated on the steam bath for 1 h. The solution was cooled and the solid that separated was recovered by filtration and dried. This solid was added to a solution made up of benzene (50 ml), ethanol (20 ml), and concentrated H_2SO_4 (0.5 ml), and the solution was then heated at reflux for 24 h. The cooled reaction mixture was poured into water (200 ml) and extracted with $CHCl_3$ (2 × 100 ml). The organic layer was separated, dried over Na_2SO_4, and evaporated in vacuo. There was obtained 2.4 g (56%) of **6** as a white solid, mp 90–92 °C. A sample was crystallized for analysis from *t*-BuCl; the melting point did not change. Anal. ($C_{10}H_{20}N_2O_3$) C, H, N.

(b) Ethyl 7-Cyanamidoheptanoate (7). To a stirred solution of **6** (21 g, 100 mmol) in pyridine (100 ml) was added *p*-toluenesulfonyl chloride (20 g, 110 mmol) in one portion. The solution was stirred at room temperature for an additional 3 h. Most of the solvent was removed in vacuo, water (200 ml) was added, and the reaction was extracted with ethyl acetate (2 × 150 ml). The organic layer was washed with 5% HCl to remove traces of pyridine and then with brine and dried over Na_2SO_4. Evaporation of the solvent gave pure **7** (16.0 g, 81%) as a viscous oil. Anal. ($C_{10}H_{18}N_2O_2$) C, H, N.

(c) Ethyl 7-[*N*-(4-Acetoxynonyl)cyanamido]heptanoate (8a). Ethyl 7-cyanamidoheptanoate (**5**) (1.98 g, 10 mmol) was added, during 30 min, to a stirred suspension of NaH (240 mg, 10 mmol) (50% in mineral oil) in benzene (20 ml) and DMF (20 ml). After 1 h of additional stirring 1-chloro-4-acetoxynonane (2.20 g, 10 mmol) was added and stirring was continued for 20 h at room temperature. The reaction mixture was poured into water (100 ml), carefully acidified with dilute HCl, and then extracted with benzene. The benzene layer was washed with brine and dried over Na_2SO_4, and then the solvent was removed in vacuo. The residue was purified by chromatography on silica gel with 5% MeOH in $CHCl_3$ as eluent. There was obtained 3.5 g (92%) of **8a** as an oil. Anal. ($C_{21}H_{38}N_2O_4$) H, N; C: calcd, 65.96; found, 65.53.

(d) 7-[1-(4-Hydroxynonyl)ureido]heptanoic Acid (9a). A solution of the ester **8a** (1.8 g, 4.7 mmol) and NaOH (680 mg, 17 mmol) in water (10 ml) and ethanol (40 ml) was let stand at room temperature for 48 h. Water (50 ml) and then 30% H_2O_2 (1.25 ml) were added and the solution was let stand for an additional 1 h to effect the hydrolysis of the cyano function. The solution was diluted further with water (75 ml), acidified with dilute HCl, and extracted with CH_2Cl_2 (4 × 50 ml). The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated in vacuo. Trituration of the residue with ether afforded solid **9a** (1.4 g, 87%), mp 69–70 °C. Crystallization from ether gave material with mp 75–76 °C: NMR δ 0.90 (3 H, t, CH_2CH_3), 5.75 (2 H, m, NH_2), 7.80 (2 H, m, COOH, OH). Anal. ($C_{17}H_{34}N_2O_4$) C, H, N.

7-[1-(4-Hydroxy-2-nonenyl)ureido]heptanoic Acid Hydrate (9b). (a) Ethyl 7-[*N*-(4-Acetoxy-2-nonenyl)cyanamido]heptanoate (8b). To a stirred suspension of NaH (528 mg, 22 mmol) in benzene (20 ml) and DMF (20 ml) was added **7** (4.0 g, 20 mmol). After 1 h of additional stirring, 1-bromo-4-acetoxy-2-nonene[1] (5.8 g, 22 mmol) was added and stirring was continued for 20 h at room temperature. Work-up as described for com-

pound 8a above afforded 5.7 g (75%) of 8b as an oil. Anal. ($C_{21}H_{36}N_2O_4$) C, H, N.

(b) 7-[1-(4-Hydroxy-2-nonenyl)ureido]heptanoic Acid Hydrate (9b). Using exactly the procedure described for compound 9a there was converted 5.7 g (15 mmol) of **8b** into the subject compound. The product was purified by chromatography on silica gel using 10% MeOH in $CHCl_3$ as eluent. There was obtained 2.0 g (38%) of **9b** as a viscous oil. Anal. ($C_{17}H_{32}N_2O_4 \cdot H_2O$) C, H, N.

7-[1-(4-Hydroxy-4-methylnonyl)ureido]heptanoic Acid (9c). (a) Ethyl 7-[*N*-(4-Acetoxy-4-methylnonyl)cyanamido]heptanoate (8c). The ester **7** (4.0 g, 20 mmol) was alkylated in the same manner as described for **8a** using NaH (528 mg, 22 mmol) and 1-chloro-4-acetoxy-4-methylnonane (**13**, 5.15 g, 22 mmol). The same work-up afforded 5.2 g (62%) of **8c** as an oil. Anal. ($C_{22}H_{40}N_2O_4$) C, H, N.

(b) 7-[1-(4-Hydroxy-4-methylnonyl)ureido]heptanoic Acid (9c). Compound **8c** was converted to the subject compound by the use of the procedure described for 9a. The product was purified by chromatography on silica gel using 10% CH_3OH in $CHCl_3$ as eluent. The product was a viscous oil. Anal. ($C_{18}H_{36}N_2O_4$) C, H, N.

7-[*N*-(4-Hydroxynonyl)cyanamido]heptanoic Acid Hemihydrate (10). A solution of ester 8a (3.0 g, 7.8 mmol) and NaOH (1.4 g, 35 mmol) in water (10 ml) and ethanol (50 ml) was let stand at room temperature for 48 h. The same work-up described for compound **5** afforded 1.5 g (60%) of **10** as a viscous oil. Anal. ($C_{17}H_{32}N_2O_3 \cdot 0.5H_2O$) C, H, N.

7-[1-(4-Hydroxynonyl)thioureido]heptanoic Acid (11). A solution of **10** (4.2 g, 13.4 mmol) in thiolacetic acid (15 ml) was stirred gently while being irradiated with uv light for a period of 2.5 h. The solution was let stand for 20 h at room temperature and then the solvent was removed in vacuo. The residue was taken up in NH_4OH (150 ml) and stirred at room temperature for 48 h. The cooled solution was carefully acidified with dilute HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by chromatography on silica gel using 6% MeOH in $CHCl_3$ as eluent. There was obtained 3.2 g (71%) of **11** as a viscous oil. Anal. ($C_{17}H_{34}N_2O_3S$) C, H, N.

1-Chloro-4-acetoxy-4-methylnonane (13). To the Grignard reagent prepared from 1-bromopentane (4.8 g, 40 mmol) and magnesium (0.96 g, 40 mmol) in ether was added 5-chloro-2-pentanone (6.0 g, 40 mmol). The reaction mixture was stirred at room temperature for 1 h and then cooled to 15 °C. Acetic anhydride (6 ml, excess) was added carefully and the ether solution was let stand for 20 h. Water was added; the ether layer was separated, washed with brine, and dried over Na_2SO_4. Distillation afforded the product: 4.3 g (46%); bp 88 °C (0.1 mm). Anal. ($C_{12}H_{23}ClO_2$) C, H.

Biological. Mouse Ovary Prostaglandin Assay.[7] 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 μCi of adenine-8-^{14}C. The tissues were incubated 1 h at 37 °C with moderate shaking to cause a pool of intracellular ATP-^{14}C 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 Me_2SO. 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 μl of a nucleotide mixture solution[8] 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-^{14}C was isolated from the supernatant fluid as described by Humes and co-workers,[8] including the final paper chromatography step.

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Structure–Activity Relationship in Cinnamamides. 2.[1] Synthesis and Pharmacological Evaluation of Some (*E*)- and (*Z*)-*N*-Alkyl-α,β-dimethylcinnamamides Substituted on the Phenyl Group

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Several (*E*)- and (*Z*)-*N*-alkyl-α,β-dimethylcinnamamides variously substituted on the phenyl group were synthesized from their corresponding acids and characterized through their NMR spectra. The compounds were tested to determine the relationship existing between their action on the CNS and the activity exhibited by the corresponding amides unsubstituted on the phenyl, previously studied. Substitution with the same group always had the same effects on the biological activity of the (*E*)-*N*-alkyl-α,β-dimethylcinnamamides selected; these effects mainly regarded anticonvulsant activity which is the most noteworthy action of these compounds. This activity was reduced by electron-donating substituents and increased by electron-withdrawing ones. In the *Z* series the *p*-phenyl substitution with a halogen reduced or suppressed the CNS stimulant activity exhibited by the parent compounds.

A structure–activity relationship study[1] of a series of (*E*)- and (*Z*)-*N*-alkyl-α,β-dimethylcinnamamides showed that geometrical isomers act differently on the central nervous system; the *E* derivatives displayed CNS-depressant and anticonvulsant activity, whereas the *Z* isomers revealed CNS stimulant activity. The anticonvulsant activity,