cubated at 37° for 1 hr and then stood at 4° for 48 hr. No precipitate was observed in any tube.

Inhibition Studies. To 1-ml aliquots of rabbit antisera to poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester were added incremental amounts of up to 7000 μg of **1**. To each tube was added the equiv point amount of the antigen (30 μg) and the tubes were then incubated at 37° for 1 hr. After standing at 4° for 48 hr, the precipitates were collected, washed twice with buffer (0.05 *M* K_2HPO_4–NaOH), pH 7.0, and collected by centrifugation. The total amount of protein precipitated in each tube was determined by N analysis by a micro-Kjeldahl method to give 105 μg of protein N for each tube. A control run simultaneously with the preceding experiment but not containing any added polypeptide **1** gave a protein N analysis of 105 μg. Therefore, the precipitin reaction between poly(Tyr-Glu-Ala-Gly)Gly-*l*-^{14}C Et ester and its antisera was not inhibited by the addition of up to 7000 μg of **1**.

Acknowledgments. This work was supported by the National Science Foundation, the Hartford Foundation and the State of Alabama.

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exo-Bicyclo[3.1.0]hexane-6-carboxylic Acid and Related Compounds, Oral Hypoglycemic Agents

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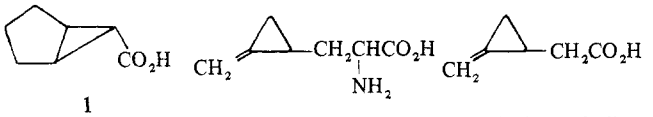
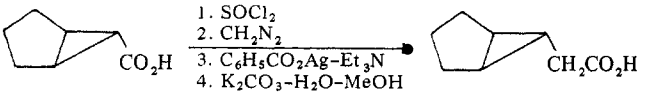
Screening for antidiabetic agents revealed that *exo*-bicyclo[3.1.0]hexane-6-carboxylic acid (**1**)† possessed good hypoglycemic activity in the glucose-primed, fasted, intact rat. This compound is structurally related to the hypoglycemic compounds, Hypoglycin A and its biological metabolite[2] (for a good review of Hypoglycin and Hypoglycin-like compounds see ref 3). However, although Hypoglycin-type



1 Hypoglycin A biological metabolite of Hypoglycin A

compounds produced an increase in plasma-free fatty acids (FFA's),[4] **1** caused a decrease in FFA's in the fasted rat.‡ This observation, coupled with the fact that **1** is relatively nontoxic (as opposed to Hypoglycin A),[5] suggested that these 2 types of compounds act by different mechanisms to lower blood sugar. A study aimed at obtaining insight into the various structural features necessary for hypoglycemic activity in this new class of hypoglycemic agents was made.

Chemistry. Acids **1**,[1] **2**,[6] **4**,[7] **7**,[1] **8**,[8] and **9**[9] were prepared by published procedures or slight modifications thereof. Compd **3** was obtained by the hydrolysis of the corresponding ethyl ester.[10] Compd **10** was formed by application of the Arndt-Eistert reaction sequence to acid **1**.



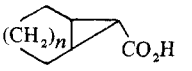
1 **10**

The esters in Table III and the amides in Table IV were prepared from the appropriate acid chlorides (see Experimental Section).

Structure-Activity Relationship Considerations. The bicyclic ring system in **1** appears to be necessary for activity in that cyclopropanecarboxylic acid (**5**) and cyclohexanecarboxylic acid (**6**) were both inactive at the highest doses tested. The exo configuration is required in that the corresponding endo isomer (**7**) and two of its amides, **15** and **16**, were inactive while the corresponding exo isomers (**1**, **17**, and **21**) were active. Variations in the size of the larger ring (see Table I) resulted in **2** which possessed substantially greater activity than the initial lead (**1**). A spirocyclopropane analog (**8**) and a tricyclic analog (**9**) possessed no or substantially reduced activity.

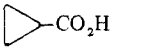
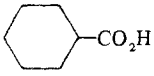
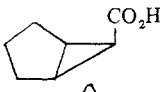
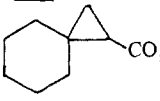
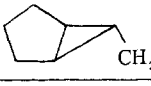
Analog **10**, in which CO_2H is separated from the cyclopropyl ring by CH_2 (as in the active metabolite of Hypoglycin A),[2] possessed good activity. This was surprising in

Table I

No.	*n*	Lowest dose with consistent activity, mg/kg[a]
		
1	1	6.25
2	2	2.0
3	3	100
4	4	>100

[a] Lowest dose of tolbutamide with consistent hypoglycemic activity = *ca.* 25 mg/kg.

Table II

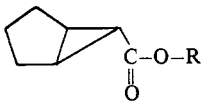
No.	Structure	Lowest dose with consistent activity, mg/kg[a]
5[b]		>100
6[b]		>100
7		>100
8		>100
9		100
10		12.5

[a] Lowest dose of tolbutamide with consistent hypoglycemic activity = *ca.* 25 mg/kg. [b] Aldrich Chemical Company, Inc., Milwaukee, Wis.

†This compd was first prepd at The Upjohn Company by E. S. Cerda according to ref 1.

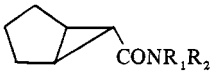
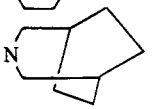
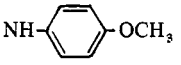
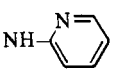
‡Unpublished work by G. C. Gerritsen, The Upjohn Company.

Table III

No.	R	Bp (mm), °C	Formula	Lowest dose with consistent activity, mg/kg[a]
				
11	$CH_2(CH_2)_6CH_3$	110–116 (0.5–1)	$C_{15}H_{26}O_2$	12.5
12	$C(CH_3)_2CH_2CH_3$	57 (0.05)	$C_{12}H_{20}O$	25
13	$CH_2CH{=}CHC_6H_5$ (trans)	149–153 (0.07)	$C_{16}H_{18}O_2$	12.5
14	$CH_2C{\equiv}CH$	68–70 (0.05)	$C_{10}H_{12}O_2$	6.25

[a]Lowest dose of tolbutamide with consistent hypoglycemic activity = *ca.* 25 mg/kg.

Table IV

No.	Stereochem	NR_1R_2	Mp, °C	Recrystn solvent	Formula	Lowest dose with consistent activity, mg/kg[a]
						
15	Endo	NH_2	125.5–127	$CHCl_3$–hexane	$C_7H_{11}NO$	>100
16	Endo		112–113	Hexane	$C_{12}H_{14}N_2O$	>100
17	Exo	NH_2	198–199	$CHCl_3$	$C_7H_{11}NO$	25
18	Exo		79–80	Hexane	$C_{12}H_{19}NO$	>100
19	Exo		86.5–87.5	Hexane	$C_{15}H_{23}NO$	>100
20	Exo		180.5–181	Hexane	$C_{14}H_{17}NO_2$	100
21	Exo		122–123.5	Hexane	$C_{12}H_{14}N_2O$	6.25

[a]Lowest dose of tolbutamide with consistent hypoglycemic activity = *ca.* 25 mg/kg.

that previous studies with Hypoglycin-type compounds had suggested that a C–C double bond is necessary for hypoglycemic activity.[11] Either the original suggestion is incorrect or **10** is acting by a different mechanism.

All esters and all primary and secondary amides of **1** possessed hypoglycemic activity; however, none were more active than the free acid **1**. Two tertiary amides of **1** (**18** and **19**) were inactive at the highest doses tested.

Experimental Section[§]

Biological Testing. Glucose-primed, fasted (18–24 hr), Upjohn Sprague-Dawley, pathogen-free, male rats were the test animals. The test compd was administered orally at various dosages in 0.5 ml of sterile vehicle (6 rats/group). Immediately following administration of the test material, the animals were injected sc with 125 mg of glucose in 1 ml of 0.9% saline. Two hours later the rats were bled, *via* the vena cava, while under 5-allyl-5-(2-cyclopenten-1-yl)barbituric acid anesthesia and blood glucose concns were detd by Auto-Analyzer, which utilized a modification of a method described by Hoffman.[12] The activity of the test compd is expressed as the lowest dose which consistently produced a significant hypoglycemic response.

exo-**Bicyclo[5.1.0]octane-8-carboxylic Acid (3).** Ethyl *exo*-bicyclo[5.1.0]octane-8-carboxylate[10] was hydrolyzed with KOH in H_2O–EtOH to afford **3**, mp 140.5–142° (hexane). *Anal.* ($C_9H_{14}O_2$) C, H.

exo-**Bicyclo[3.1.0]hexane-6-acetic Acid (10).** *exo*-Bicyclo[3.1.0]hexane-6-formyl chloride (5.8 g; 0.040 mole) (prepd from **1** and $SOCl_2$) was added dropwise to a stirred, cooled (0–5°) soln of CH_2N_2 (*ca.* 0.20 mole) in Et_2O (300 ml). After an addnl 30 min the soln was covered and allowed to stand at room temp for 3 days. The solvent and excess CH_2N_2 were removed on a rotary evaporator, and the residue was dissolved in abs MeOH and was treated with a freshly prepd soln of silver benzoate (4.0 g) in Et_3N (40 ml). The mixt was stirred for 1 hr and then allowed to stand for 2 days. It was filtered, and the solvent was removed from the filtrate on a rotary evaporator. The residue was dissolved in Et_2O (400 ml) and washed with H_2O (2 × 250 ml), 1 *N* HCl (2 × 250 ml), and satd $NaHCO_3$ (2 × 400 ml). The soln was dried ($MgSO_4$) and concd on a rotary evaporator. The residue was dissolved in a mixt of EtOH (10 ml) and 20% K_2CO_3 (40 ml) and heated at reflux with stirring for 2 hr. The mixt was cooled, dild with H_2O (200 ml), and washed with Et_2O (2 × 200 ml). The aq layer was acidified with 2.5 *N* H_2SO_4 and extd with Et_2O (2 × 150 ml). The combined exts were dried ($MgSO_4$), and the solvent was removed on a rotary evaporator. The residue was vacuum distd to afford **10** (20% overall yield), bp 82° (0.1 mm). *Anal.* ($C_8H_{12}O_2$) C, H.

exo-**Bicyclo[3.1.0]hexanecarboxylic Acid Esters (11, 13, and 14).** *exo*-Bicyclo[3.1.0]hexane-6-formyl chloride (11.4 g; 0.079 mole) was added dropwise to a stirred, cooled (0–5°) soln of the appropriate alc (0.072 mole) and pyridine (6.25 g; 0.079 mole) in C_6H_6 (100 ml). The mixt was stirred an addnl 2 hr at room temp and dild with H_2O (200 ml). The sepd org layer was washed with satd NH_4Cl (150 ml) and satd $NaHCO_3$ (150 ml) and dried (Na_2SO_4). The solvent was removed on a rotary evaporator and the residue was vacuum distilled (see Table III).

exo-**Bicyclo[3.1.0]hexane-6-carboxylic Acid, *tert*-Pentyl Ester (12).** *exo*-Bicyclo[3.1.0]hexane-6-formyl chloride (11.4 g; 0.079 mole) was added dropwise to a stirred, refluxed soln of *tert*-pentyl alcohol (6.3 g; 0.072 mole) and $Me_2NC_6H_5$ (9.6 g; 0.079 mole) in Et_2O (100 ml). The mixt was heated at reflux for an addnl 30 hr and was worked up as above to afford **12** (see Table III).

Bicyclo[3.1.0]hexane-6-carboxamides (15–21). The appropriate bicyclo[3.1.0]hexane-6-formyl chloride (11.4 g; 0.079 mole) (from **1** or **7** and $SOCl_2$) in $CHCl_3$ (50 ml) was added dropwise to a stirred soln of the amine (0.087 mole) and Et_3N (8.8 g; 0.087 mole) in $CHCl_3$ (100 ml). The mixt was stirred for an addnl hour and then washed with satd NH_4Cl (100 ml) and satd $NaHCO_3$ (100 ml) and dried (Na_2SO_4). The solvent was removed on a rotary evaporator and the residue was recrystd (see Table IV).

§ All melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The structures of all compds were supported by ir and nmr spectra and, in many cases, by mass spectra. Ir spectra were obtd on a Perkin-Elmer Model 421 recording spectrometer in Nujol mulls, the nmr spectra were recorded on a Varian-A-60A spectrometer, and the mass spectra were detd on an Atlas CH-4 spectrometer. All compds were analyzed for C, H, and N (where applicable); in each case results were within ±0.4% of the theoretical values.

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Estradiol Analogs with Conformational Flexibility. 4-(1,2,3,4-Tetrahydro-6-hydroxy-2-naphthyl)-butan-2-ol†

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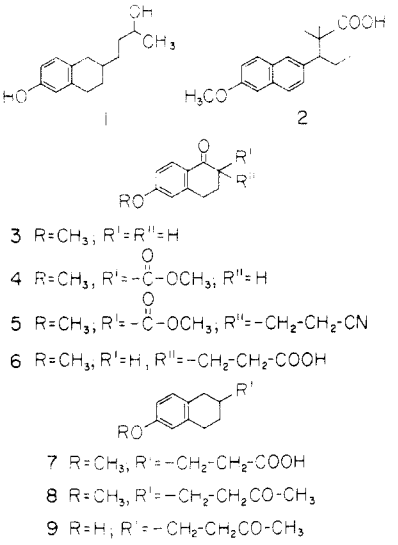
One approach to the development of nonsteroidal estrogens is through simple analogs of estradiol lacking parts of the C and D rings. We have therefore synthesized and studied the biological activity of 4-(1,2,3,4-tetrahydro-6-hydroxy-2-naphthyl)butan-2-ol (**1**). Horeau[1] has already shown that naphthalene derivatives with side chains extending from C-2, such as methallenestril (**2**), can retain strong estrogenic activity. Our model compound **1** was anticipated to have the conformational flexibility to bind selectively to some estradiol receptors, and possibly to show at least a partial separation of estrogenic and other hormonal (*e.g.*, hypocholesterolemic) activities. Furthermore, the synthetic route developed for **1** would allow the introduction of other substituents with ease, thus making available a variety of bicyclic derivatives for additional study.

The synthesis of **1** was straightforward. The carbomethoxy derivative **4**,[2] obtained from 6-methoxy-1-tetralone (**3**), was cyanoethylated[3] with acrylonitrile in the presence of Triton B to give the cyanoethyl compound **5** in good yield. Hydrolysis of **5** with a mixture of HCl and AcOH resulted in the propionic acid **6**. A modified Wolff-Kischner reduction[4] of the keto acid **6** yielded **7**, which on subsequent treatment with MeLi gave the methyl ketone **8**. This methoxy compound **8** was then demethylated by fusion with pyridine hydrochloride at 205° to give the phenol **9**. The carbonyl group in the side chain was finally reduced with $NaBH_4$ to furnish the desired alcohol **1**. All the compounds described were racemates, but the final reduction introduced a second asymmetric center into the molecule. There was no obvious steric control of this reaction; this, together with the yield (69%) and the single crystallization of the chromatographically pure product, make it improbable that the compound tested was a single (inactive) diastereoisomer and that the other might be active.



Compound **1** was assayed for both estrogenic and antiestrogenic activity in rats, using uterine weight and vaginal opening as criteria.‡ It was found inactive at doses up to 300 μg/kg body weight. It was subsequently assayed for hypocholesterolemic activity in rats at 7 mg/kg twice daily for 2 days, and the serum cholesterol was determined.§ No significant lowering of the cholesterol level was observed.

Experimental Section#

Methyl 1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthalenecarboxylate (4) was prepared from **3** as described by Juday *et al.*;[5] the physical constants were in agreement with those described by Bachmann and Thomas.[2]

Methyl 2-Cyanoethyl-1,2,3,4-tetrahydro-6-methoxy-1-oxo-2-naphthalenecarboxylate (5). To the carbomethoxy-1-tetralone **(4)** (10 g) in *p*-dioxane (10 ml, free from peroxides) were added acrylonitrile (3 ml) and Triton B (0.9 ml, 40% in MeOH), and the mixt was stirred at ambient temp for 16 hr. The reaction product was isolated** with Et_2O and recrysted from a mixt of Et_2O and petr ether to yield 7.5 g (62%) of **5**: mp 66–69°; 231 (ϵ 5920) and 280 mμ (15,000). *Anal.* ($C_{16}H_{17}NO_4$) C, H, N.

†Supported in part by a research grant from Mallinckrodt Chemical Works, St. Louis, Mo.

‡Estrogen and antiestrogen assays were conducted under the direction of Dr. Elva G. Shipley at the Endocrine Laboratories, Madison, Wis.

§The serum cholesterol levels were determined at the Southwest Foundation for Research and Education by Mr. Armando de la Peña.

#Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were recorded in KBr discs on a Unicam S.P. 200 spectrometer and were compatible with the structures assigned. Neutral aluminum oxide, activity II, was employed for column chromatography. Petroleum ether refers to that fraction boiling from 40–60°. The compounds described are all racemates. Microanalyses were performed by Micro-Tech Laboratories, Skokie, Ill. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

**The following sequence describes a typical isolation procedure. The reaction mixture was treated with H_2O and extracted with the specified organic solvent. The solvent extract was washed with brine, and dried over Na_2SO_4, and filtered, and the solvent was evapd under reduced pressure on a Büchi rotary evaporator at 60–65°. The residue left in the flask was then purified as described.