desired **12b**: mp 168–170 °C; NMR δ 0.89 (s, 3 H, C_{18} H), 2.3 (s, 3 H, $SeCH_3$), 6.78–7.25 (m, 3 H, Ar H); MS, *m/e* 365 (M^{+1} for ^{80}Se), 363 (M^{+1} for ^{78}Se), 347 ($M^{+1} - H_2O$), 271 ($M^{+1} - SeCH_3$). Anal. ($C_{19}H_{24}O_2Se$) C, H.

16α-(Phenylseleno)-17β-estradiol (13). Compound **10a** (509 mg, 1 mmol) was dissolved in a minimum amount of anhydrous ether (15 mL). This solution was slowly added to a precooled (−15 °C) suspension of $LiAlH_4$ (75 mg, 2 mmol) in ether (20 mL) under nitrogen. The reaction mixture was stirred at −15 °C for 2 h and then for another hour at room temperature. Three drops of 50% NaOH was added. The suspension was diluted with ether and filtered. Ether was evaporated, and the residue was dissolved in 95% ethanol (50 mL) and water (10 mL). To this solution was added 0.15 g of *p*-toluenesulfonic acid. After 1 h at 50 °C, 200 mL of water was added. The solution was allowed to cool to room temperature and extracted with ether. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and filtered, and the filtrate was evaporated to dryness. Crystallization from benzene/hexane provided **13** as a fluffy mass of fine crystals (50% overall yield): mp 233–234 °C; NMR δ 0.77 (s, 3 H, C_{18} H), 1.55 (s, 1 H, C_{17} OH), 4.05 (m, 1 H, C_{16} H), 4.6 (s, 1 H, C_3 OH), 6.55–7.61 (m, 8 H, Ar H); MS, *m/e* 429 (M^{+1} for ^{80}Se), 427 (M^{+1} for ^{78}Se), 411 ($M^{+1} - H_2O$), 273 (M^{+2} – SePh), 255 ($M^{+2} - H_2O$ – SePh). Anal. ($C_{24}H_{28}O_2Se$) C, H.

Competitive Binding Assay Using Immature Rat Uterus Cytosol. Immature female Sprague–Dawley derived rats (21–25 days old) were killed by cervical dislocation. The uteri were removed, cleaned from adhering fat and mesentery, and placed in cold 0.9% NaCl. The uteri (2 uteri/mL) were homogenized at 4 °C in TEE buffer (10 mM Tris, 1.5 mM Na_2EDTA, and 1 mM dithiothreitol; pH 7.4 at 4 °C) in a motor-driven all-glass conical tissue homogenizer. The homogenizing vessel was held in an ice bath during the homogenization. The homogenate was centrifuged at 4 °C for 10 min. The fat-free supernatant was mixed with TEE buffer to provide a concentration equivalent to 1 uterus/mL.

An accurately weighed sample of nonradioactive competitor (25 mg) was dissolved in 25 mL of absolute ethanol. A 10-μL aliquot of this solution was diluted to 10 mL with TEE buffer to give a 1-μg/mL stock solution. Serial dilutions with TEE buffer were prepared to give concentrations ranging from 2.5 to 200 ng/0.2 mL. For cold estradiol and ethynylestradiol, the range in concentration was 0.5 to 200 ng/0.2 mL.

Microfuge tubes (1.5-mL capacity) were cooled on ice. To each tube was added 25 μL of a 2×10^{-7} M [^{3}H]estradiol solution in TEE buffer, followed by 200-μL aliquots of the competitor solutions, and the tubes were vortexed. After addition of 0.5 μL of cytosol to each tube, the tubes were again vortexed and placed on ice in a refrigerator. After 20 h, the incubation was terminated by the addition of 150 μL of a well-mixed cold dextran–charcoal suspension to each tube. These tubes were again vortexed and placed on ice in a refrigerator. After 10 min, the charcoal was spun down for 5 min. The last step was repeated with all the supernatant. The 250-μL aliquots of the supernatant were pipetted into scintillation vials containing 10 mL of Aquasol-2 (New England Nuclear). The radioactivity was measured in a liquid scintillation counter for a time that would give less than 2% counting error at the 95% confidence level.

17β-[6,7-^{3}H(N)]Estradiol, specific activity 53.0 Ci/mmol, was obtained from New England Nuclear. The radiochemical purity was greater than 98% when determined by TLC on silica gel G with the solvent system benzene/ethanol (9:1, v/v). Spots were located by autoradiography on X-Omat TL film (Eastman Kodak).

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Synthesis and Topical Antiinflammatory Activity of Some Steroidal [16α,17α-*d*]Isoxazolidines

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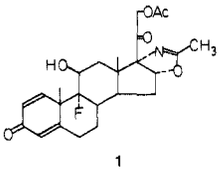
1,3-Dipolar cycloaddition of *N*-methylnitrone, *N*-benzylnitrone, and pyrroline *N*-oxide to 1,4,16-pregnatriene-3,20-diones is described. In each case only [16α,17α-*d*]isoxazolidines were isolated. The pentacyclic adducts **16–19** were active topical antiinflammatory agents in mice, with **18** being more potent than any of the standard compounds tested. The hexacyclic adduct **20** was inactive in this assay.

The fusion of heterocyclic rings onto steroid nuclei in order to alter the biological activity of the parent molecule has been a very productive endeavor for medicinal chemists. This is particularly true for the antiinflammatory steroids where several such analogues have found clinical use. Examples of corticosteroids with ring D fused heterocycles include the [17α,16α-*d*]-2′-methyloxazoline **1**[2] and the 16α-hydroxy-16,17-acetonides **2** and **3**. These compounds are potent, topical antiinflammatory agents in man,[3] and the acetonides in particular are widely used clinically for a variety of skin diseases.

In a program aimed at finding novel compounds with high topical antiinflammatory activity, we have sought to synthesize steroids with other heterocyclic rings fused to the 16,17-positions of corticosteroids. In this report we



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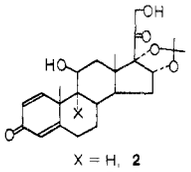

X = H, **2**
X = F, **3**

R = CH_3, **4**
R = CH_2Ph, **5**

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describe the preparation and topical antiinflammatory activity of a series of [16α,17α-*d*]isoxazolidines. We chose

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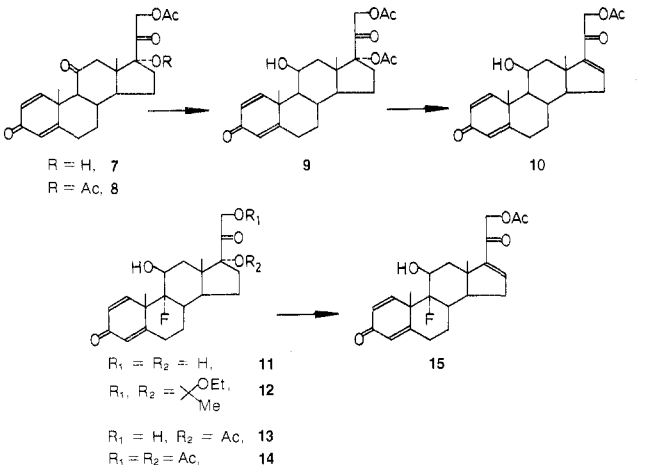
Scheme I



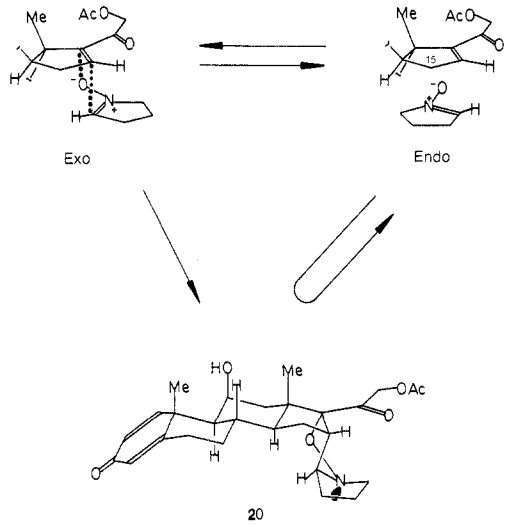
this particular heterocycle because it combines two structural features of high-potency, systemic corticosteroids (an oxygen function at C_{17} and a one-carbon fragment at C_{16}) with a heterocyclic ring fused at C_{16} and C_{17}, which is a feature of the topically active group of compounds exemplified by **1–3**.

We envisaged that the desired [16α,17α-*d*]isoxazolidines would be formed by 1,3-dipolar cycloaddition reactions of the nitrones **4–6** to the 16,17 double bond of suitably substituted 16-pregnen-20-ones. Culbertson, Moersch, and Neuklis[4] reported that the addition of *C,N*-diphenylnitrone to 16-dehydropregnenolone acetate gave both the [16α,17α-*d*]- and the [17α,16α-*d*]isoxazolidines.[5] However, there have been no reports of such additions with C-unsubstituted nitrones, and at the outset it was not at all certain how the decrease in steric bulk at the C end of the dipole would influence the regiochemistry of this reaction.

Chemistry. Scheme I shows the preparation of the starting 1,4,16-pregnatriene-3,20-diones **10** and **15**. Acetylation of prednisone 21-acetate (**7**) [$(CF_3CO)_2O$, *p*-TSA, HOAc], followed by selective reduction[6] ($NaBH_4$) of the 11-ketone, gave prednisolone 17,21-diacetate (**9**). Treatment of **9** with KOAc in DMF at 100 °C[7] then afforded **10** (20% from **8**). 9α-Fluoroprednisolone **11** formed the corresponding 9α-fluoro derivative **15** via the 17,21-ethyl orthoacetate **12**; acid hydrolysis of **12** to **13**, followed by acetylation to **14** and elimination of the 17-acetate (KOAc, DMF), led to **15** (16% from **11**).

1,3-Dipolar cycloaddition of *N*-methylnitrone (**4**) to **10** gave a single adduct, **16**, isolated in 40% yield by column chromatography.[8] Mass spectroscopy and microanalysis indicated that **16** was a monoadduct, while the ^1H and ^{13}C NMR spectra clearly showed that reaction had occurred at the 16,17 double bond, since the signals due to the cross-conjugated dienone system in ring A were still present. Compound **16** was the [16α,17α-*d*]isoxazolidine because its ^1H NMR spectrum showed only one signal for a methine proton geminal to oxygen (the 11α-H signal at 4.43 ppm). A spectrum of the regioisomeric [17α,16α-*d*]-isoxazolidine would have shown two such signals. This assignment was confirmed by ^{13}C NMR spectroscopy; the spectrum of **16** showed C_{17} at 99.97 ppm and C_{16} at 48.10 ppm, downfield from their usual positions by 9 and 12 ppm, respectively, due to the deshielding effect of the nitrogen atom. These two signals appeared as singlet and doublet, respectively, in the SFOR spectrum, which can only be accommodated by the assigned structure. The orientation of the isoxazolidine ring was assigned as 16α,17α by analogy to the known direction of attack of other dipolarophiles to the 16,17 double bond.[4,9] In addition, the ^1H and ^{13}C NMR spectral data were clearly in accord with this assignment.

Scheme II



N-Benzylnitrone and **10** gave the analogous isoxazolidine **17**, and cycloaddition of *N*-methyl- and *N*-benzylnitrones to **15** produced the 9α-fluoro analogues **18** and **19**, respectively. Crystallization or chromatography isolated only the [16α,17α-*d*]isoxazolidines in each of these three cases. Pyrroline *N*-oxide[10] **6** on reaction with **10** yielded adduct **20** exclusively, forming three contiguous chiral centers at once. The ^1H NMR spectrum of **20** showed the expected signals, although the 3 H signal (δ 3.4) of the protons α to nitrogen was broad. Poor resolution of this signal prevented determination of the $C_{3'}$ stereochemistry, but inspection of models suggested that $C_{3'}$ should be *R*. This assignment is explained in Scheme II. Aligning stereomodels of the dipole **6** and the dipolarophile **10** in parallel planes[11] simulates exo and endo transition states for kinetically controlled cycloadditions.[12,13] Inspection of the endo transition state reveals a severe steric interaction between the 14α hydrogen of **10** and one of the C_4 hy-

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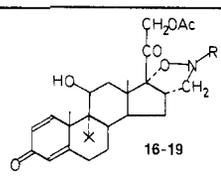
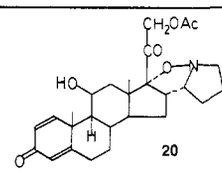
Table I. Physical and Biological Data for Steroidal [16α,17α-*d*]Isoxazolidines and Standard Steroids





Compd	X	R	Yield (%)	Mp (°C)	$[\alpha]_D^{25}$ [a]	λ_{max} (nm(ε)) [b]	Potency [c]
16	H	Me	40	222-5	−14.3	242 (15,000)	75 (36-114)
17	H	CH_2Ph	33	133.5-7	−200.0	242 (15,000)	69 (26-112)
18	F	Me	35	237-40	−133.8	238 (15,600)	156 (129-183)
19	F	CH_2Ph	24	167-8	+192.2	238 (15,900)	62 (57-67)
20	—	—	43	209-12	+37.5	240 (14,800)	Inactive [d]
1	—	—	—	—	—	—	104 (23-185)
2	—	—	—	—	—	—	102 (85-119)
3	—	—	—	—	—	—	117 (83-151)

[a] In DMF. [b] In MeOH. [c] Acute, mouse ear, croton oil assay where betamethasone valerate = 100. Numbers in parentheses are 95% confidence level intervals of the estimated potencies. [d] 0.3 μg was the highest dose tested.

drogens of **6**. This destabilizing interaction should slow any cycloaddition, yielding the $C_{3'}$ *S* epimer of **20** relative to that forming **20**, since the corresponding exo transition state lacks such destabilizing steric interactions.

Biological Results

Topical antiinflammatory activity was measured in mice by a modification[14] of the croton oil ear assay of Tonelli et al.[15] The isoxazolidines **16–19** were found to be active as topical antiinflammatory agents in this acute assay (Table I). In fact, the most potent compound, **18**, was considerably more potent than the standard, betamethasone 17-valerate, as well as the other 16,17-fused heterocyclic steroids **1–3**. It is interesting to note that in this [16α,17α-*d*]isoxazolidine series the introduction of the fluorine at C_9 doubles the topical potency of the 2′-methyl compound **16** but has no effect upon the 2′-benzyl analogue **17**. The hexacyclic compound **20**, derived from pyrroline *N*-oxide was, however, inactive at the highest dose tested; presumably, interaction of ring D and the substituents at 16α and 17α with some biological receptor is hindered by the bulk of the pyrrolidine ring.

Experimental Section

Melting points were taken on a Fisher Digital melting point analyzer Model 355 and are uncorrected. ^1H and ^{13}C NMR spectra were obtained on Varian CFT-20 and XLFT-100 instruments, respectively, in $CDCl_3$ solution with Me_4Si as an internal standard. Silica gel preparative (2000 μm) and analytical (250 μm) thin-layer chromatography (TLC) plates were obtained from Analtech, Inc., and the silica gel used for column chromatography was TLC grade supplied by E. Merck (silica gel G-60).

11β,21-Dihydroxy-1,4,16-pregnatriene-3,20-dione 21-Acetate (10). Trifluoroacetic anhydride (125 mL) was added over 20 min to a cooled (15 °C), stirred solution of prednisone acetate (**7**; 50 g) and *p*-TSA·H_2O (5 g) in glacial AcOH (125 mL). After stirring for 1.5 h at 15 °C and for 2 h at room temperature, it was poured into ice–water (3 L). The precipitate was filtered off, thoroughly washed with water, air-dried, and crystallized from Me_2CO/hexane to give **8** (37 g). A portion of **8** (32 g) was dissolved in THF (150 mL) and MeOH (150 mL) at 0 °C, and $NaBH_4$ (0.95 g) was added with stirring. After 1 h at 0 °C, the reaction mixture was poured into ice-cold 1 N HCl, and the precipitate was filtered off, washed with water until neutral, and dried in vacuo to give **9** (26 g): crystallization from Me_2CO/hexane gave pure **9** (17 g).

Compound **9**, 16 g, with anhydrous KOAc in DMF (120 mL) was heated to 100 °C for 18 h. The reaction mixture was then poured into ice–water, and the product was extracted into $CHCl_3$. The organic extract was washed with water and dried (anhydrous Na_2SO_4), and the solvent was evaporated to give crude **10** (11 g), which was chromatographed on a column of silica gel (650 g). Elution with $CHCl_3$/EtOAc (3:1) gave **10** as a crystalline solid (7.1 g): mp 199–201 °C (lit.[7] mp 205–207); UV λ_{max} (MeOH) 240 nm (ε 22 500).

9α-Fluoro-11β,21-dihydroxy-1,4,16-pregnatriene-3,20-dione 21-Acetate (15). 9α-Fluoroprednisolone (**11**; 17 g) and *p*-TSA·H_2O (0.2 g) were dissolved in DMF (136 mL) and triethyl orthoacetate (936 mL). After the solution was stirred for 24 h at room temperature, pyridine (1 mL) was added, and the total reaction mixture was poured into ice–water. The product was extracted into EtOAc, and the organic extract was washed with H_2O and dried (anhydrous Na_2SO_4), and the solvent was removed under reduced pressure to give an oil comprising mainly **12**. This oil was dissolved in MeOH (600 mL), 0.1 N AcOH (3.78 mL), and 0.1 N NaOAc solution (0.42 mL) for 2 h at room temperature. The reaction mixture was concentrated under reduced pressure and extracted with EtOAc. The organic layer was washed with H_2O, dried (anhydrous Na_2SO_4), and evaporated to give **13** as a brown resin. This resin was acetylated in pyridine (150 mL) and acetic anhydride (15 mL) for 18 h at room temperature. Water (15 mL) was added, and the mixture was poured into H_2O (1.6 L). The product was extracted into EtOAc, and the organic extract was washed with H_2O, dried (anhydrous Na_2SO_4), and concentrated to an oil (15.1 g). Addition of Et_2O gave a tan precipitate of crude **14** (13 g). This product was dissolved in DMF (200 mL) and with anhydrous KOAc (9 g) was heated to 100 °C for 4 h. After cooling, the reaction mixture was poured into ice–water, and the precipitate was filtered off, washed with H_2O, and dried in vacuo to give crude **15** (8.5 g). This product was taken up in a minimum of $CHCl_3$ and filtered through a column of neutral alumina (30 g). The $CHCl_3$ eluate was concentrated to dryness (6.8 g) and then crystallized from Me_2CO/hexane to give **15** (2.6 g): mp 211–215 °C; $[\alpha]^{26}_D$ +128° (DMF); UV λ_{max} (MeOH) 238 nm (ε 24 200); ^1H NMR (Me_2SO-d_6) δ 1.17 (C_{13} CH_3, s), 1.52 (C_{10} CH_3, s), 2.08 ($OCOCH_3$, s), 4.02 (11-H, m), 4.84 and 5.12 (C_{21} H's, d, J = 18 Hz), 6.00 (C_4 H, s), 6.10 (C_2 H, dd, J = 10 and 2 Hz), 6.96 (C_{16} H, m), 7.30 (C_1 H, d, J = 10 Hz). Anal. ($C_{23}H_{27}O_5F$) C, H, F.

Steroidal 2′-Methyl-Substituted [16α,17α-*d*]Isoxazolidines 16 and 18. Compound **10** (1.5 g), *N*-methylhydroxylamine hydrochloride (0.336 g), diisopropylamine (0.436 g), and paraformaldehyde (0.076 g) in EtOH (60 mL) were heated under reflux. After 2 days, the reaction mixture was poured into H_2O, and the product was extracted into $CHCl_3$. The organic extract was washed with water, dried (anhydrous Na_2SO_4), and concentrated to an oil (1.48 g), which was chromatographed on a column of silica gel (150 g). Elution with EtOAc/$CHCl_3$ (3:7) gave first **16** (0.695 g), which crystallized from EtOAc to give pure **16** (0.48 g), and then unreacted **10** (0.072 g). **16**: ^1H NMR ($CDCl_3$) δ 0.99 (C_{13} CH_3, s), 1.46 (C_{10} CH_3, s), 2.17 ($OCOCH_3$, s), 2.62 (N-CH_3, s), 3.46 (3′-H's, m, $W_{h/2}$ = 10 Hz), 4.47 (11-H, m), 4.77 and 5.05 (C_{21} H's, d, J = 18 Hz), 6.00 (C_4 H, s), 6.25 (C_2 H, dd, J = 10 and 2 Hz), 7.27 (C_1 H, d, J = 10 Hz); ^{13}C NMR ($CDCl_3$) δ 17.0 (q, C_{18}), 20.5 (q, C_{19}), 21.1 (q, $COCH_3$), 30.6 (d, C_8), 31.0 (C_6, C_{15}), 34.1 (t, C_7), 40.8 (t, C_{12}), 44.1 (s, C_{10}), 44.4 (q, NCH_3), 45.2 (s, C_{13}), 48.1 (d, C_{16}), 50.8 (d, C_{14}), 55.5 (d, C_9), 66.3 (t, NCH_2), 67.5 (t, C_{21}), 69.9 (d, C_{11}), 100.0 (s, C_{17}), 122.5 (d, C_4), 127.8 (d, C_2), 156.3 (d, C_1), 169.9 (s, OCO), 170.6 (s, C_5), 186.5 (s, C_3), 205.1 (s, C_{20}). Anal. ($C_{25}H_{33}O_6N$) C, H, N.

Similar treatment of **15** (0.804 g) gave **18** (0.35 g), which was crystallized from EtOAc/hexane to give **18** (0.282 g): ^1H NMR (Me_2SO-d_6) δ 0.86 (C_{13} CH_3, s), 1.48 (C_{10} CH_3, s), 2.09 ($OCOCH_3$, s), 3.28 (N-CH_3, s), 3.40 (3′-CH_2, m), 4.12 (11 H, m), 4.86 (C_{21} H's, s), 5.99 (C_4 H, s), 6.10 (C_2 H, dd, J = 10 and 2 Hz), 7.27 (C_1 H, d, J = 10 Hz); ^{13}C NMR ($CDCl_3$) δ 16.5 (q, C_{18}), 20.5 (q, $COCH_3$), 23.1 (q, C_{19}), 27.7 (t, C_{15}), 31.1 (C_7 J_{CF} = 5 Hz), 31.5 (C_6), 33.5 (d, C_8, J_{CF} = 15 Hz), 37.1 (t, C_{12}), 44.0 (C_{14}, C_{16}, NCH_3), 48.17 (s, C_{13}), 48.4 (s, C_{10}, J_{CF} = 23 Hz), 66.2 (t, NCH_2), 67.6 (t, C_{21}), 71.7 (d, C_{11}, J_{CF} = 38 Hz), 99.8 (s, C_{17}), 100.5 (s, C_9, J_{CF} = 177 Hz), 125.0 (d, C_4), 129.7 (d, C_2), 152.5 (d, C_1), 166.3 (s, C_5), 170.7 (s, $COCH_3$), 186.7 (s, C_3), 205.2 (s, C_{20}). Anal. ($C_{25}H_{32}O_6NF$) C, H, N, F.

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Steroidal 2′-Benzyl-Substituted [16α,17α-*d*]Isoxazolidines 17 and 19. Compound **10** (0.768 g), *N*-benzylhydroxylamine (0.246 g), and paraformaldehyde (0.04 g) in EtOH (30 mL) was heated under reflux. After 2 days the reaction mixture was poured into H_2O, and the precipitate was filtered off, washed with water, and air-dried. This product was chromatographed on silica gel (70 g), eluting with $CHCl_3$/EtOAc (3:1) to give **17** (0.34 g), which was crystallized from Me_2CO/hexane to give pure **17** (0.191 g): ^1H NMR (Me_2SO-d_6) δ 0.82 (C_{10} CH_3, s), 1.38 (C_{13} CH_3, s), 2.06 ($OCOCH_3$, s), 3.37 (3′-H's, m), 3.84 (CH_2Ph, d, J = 4 Hz), 4.28 (11-H, m), 4.50 (C_{21} H's, s), 5.91 (C_4 H, s), 6.13 (C_2 H, dd, J = 10 and 2 Hz), 7.30 (C_1 H and phenyl H's, m); ^{13}C NMR ($CDCl_3$) δ 17.0 (q, C_{18}), 20.5 (q, C_{19}), 21.1 (q, $COCH_3$) 30.6 (d, C_8) 32.0 (C_6, C_{15}), 34.2 (t, C_7), 40.7 (t, C_{12}), 44.2 (s, C_{10}) 45.3 (s, C_{13}), 47.4 (d, C_{16}) 50.8 (d, C_{14}), 55.6 (d, C_9), 61.9 (t, NCH_2Ph), 64.1 (t, NCH_2), 67.6 (t, C_{21}), 69.8 (d, C_{11}), 99.6 (s, C_{17}), 122.5 (d, C_4), 156.4 (d, C_1), 170.0 (s, $COCH_3$), 170.5 (s, C_5), 186.5 (s, C_3), 205.0 (s, C_{20}). Anal. ($C_{31}H_{37}O_6N$) C, H, N.

Similar treatment of **15** (0.804 g) gave **19** (0.26 g), which was crystallized from EtOAc/hexane to give pure **19** (0.197 g): ^1H NMR (Me_2SO-d_6) δ 0.84 (C_{13} CH_3, s), 1.50 (C_{10} CH_3, s), 2.06 ($OCOCH_3$, s), 3.38 (3′-H's, m), 3.95 (CH_2Ph, d, J = 4 Hz), 4.14 (11-H, m), 4.52 (C_{21} H's, s), 6.00 (C_4 H, s) 6.10 (C_2 H, dd, J = 10 and 2 Hz), 7.28 (C_1 H and phenyl H's). Anal. ($C_{31}H_{36}O_6NF$) C, H, N, F.

Steroidal 2′,3′-Trimethylene-Substituted [16α,17α-*d*]-Isoxazolidine 20. Pyrroline *N*-oxide (0.204 g) and **10** (0.768 g) in EtOH (30 mL) were heated under reflux for 3 days. A further portion of **6** (0.05 g) was added, and reflux continued for a further 4 h. The reaction mixture was poured into water, and the product was extracted into EtOAc. The organic layer was washed with H_2O, dried (anhydrous Na_2SO_4), and concentrated under reduced pressure to an oil (0.619 g). Separation of this product by preparative thin-layer chromatography (development solvent $CHCl_3$) gave pure **20** (0.399 g). A portion was crystallized from $CHCl_3$/Me_2CO to give **20**: mp 209–212 °C; ^1H NMR ($CDCl_3$) δ 0.94 (C_{13} CH_3, s), 1.44 (C_{10} CH_3, s), 2.16 ($OCOCH_3$, s), 3.1–3.6 (3′-H's and pyrrolidine H's, m), 4.50 (11-H, m), 4.67 and 4.96 (C_{21} H's, d, J = 18 Hz), 5.99 (C_4 H, s), 6.22 (C_2 H, dd, J = 10 and 2 Hz), 7.22 (C_1 H, d, J = 10 Hz). Anal. ($C_{27}H_{34}O_6N$) C, H, N.

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A Specific Inhibitor of IgE-Antibody Formation: *n*-Pentyl β-D-Fructopyranoside

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n-Pentyl β-D-fructopyranoside significantly suppresses IgE-antibody formation in rats and mice when orally administered, while no formation of hemagglutinin was observed. This is the first compound that is novel in structure and which exhibits a selective inhibition of IgE-antibody formation.

By screening ethanol or water extracts of 20 traditional Chinese crude drugs that had been widely used for diseases caused by allergies to passive cutaneous anaphylaxis (PCA) and passive hemagglutination (PHA), Koda et al. found *Zizyphus fructus* to be one of the most efficient drugs.[1] An earlier experiment indicated that the ethyl α-D-fructofuranoside in the ethanol extract of *Zizyphus fructus* suppressed IgE-antibody formation in rats immunized with the dinitrophenylated *Ascaris* extract (DNP-As).[2] In order to find a potent drug, we undertook the derivatization of alkyl β-D-fructopyranosides as part of the assay of antibody formation. This report will describe how *n*-pentyl β-D-fructopyranoside given either intraperitoneally or orally effectively suppresses IgE-antibody formation in both rats and mice, without any suppression of hemagglutinin formation.

Synthesis and Characterization of Compounds 1–16. The compounds in Table I were synthesized by a modification of the method described under Experimental Section. The purity of each alkyl D-fructoside that was separated on chromatography was examined by means of gas chromatographical analysis of the trimethylsilyl derivatives; it was confirmed that each alkyl D-fructoside was 98–99% pure. The ring size and anomeric nature[3] were assigned to the glycosides by means of gas chromatography,[4] mass spectrometry,[5] and ^{13}C nuclear magnetic resonance (^{13}C NMR) determination.[6] As a potent tool, ^{13}C NMR was effectively applied to the configurational and conformational analyses. The ^{13}C NMR spectral examination of C-2 in alkyl D-fructosides clearly shows that C-2 in alkyl D-fructofuranosides resonates at a lower field than that of alkyl D-fructopyranosides. The set of resonances in methyl and ethyl D-fructofuranosides (**5** and **6**) is identified as having the β-D-furanoside form because of the cis interaction of vicinal hydroxy groups at C-2 and C-3, causing an up-field shift of the C-2 resonance relative to the same resonance of alkyl α-D-fructofuranosides (**1–4**). The set of ring carbons in alkyl β-D-fructopyranosides resonates upfield from the the corresponding set of ring carbons in alkyl D-fructofuranosides, as was demonstrated in D-fructose. As a result of ^{13}C NMR spectral studies,

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