

desired **12b**: mp 168–170 °C; NMR δ 0.89 (s, 3 H, C₁₈ H), 2.3 (s, 3 H, SeCH₃), 6.78–7.25 (m, 3 H, Ar H); MS, m/e 365 (M⁺ for ⁸⁰Se), 363 (M⁺ for ⁷⁸Se), 347 (M⁺ – H₂O), 271 (M⁺ – SeCH₃). Anal. (C₁₉H₂₄O₂Se) C, H.

16 α -(Phenylseleno)-17 β -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 LiAlH₄ (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, C₁₈ H), 1.55 (s, 1 H, C₁₇ OH), 4.05 (m, 1 H, C₁₆ H), 4.6 (s, 1 H, C₃ OH), 6.55–7.61 (m, 8 H, Ar H); MS, m/e 429 (M⁺ for ⁸⁰Se), 427 (M⁺ for ⁷⁸Se), 411 (M⁺ – H₂O), 273 (M⁺ – SePh), 255 (M⁺ – H₂O – SePh). Anal. (C₂₄H₂₈O₂Se) 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 Na₂EDTA, 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- μ L aliquot of this solution was diluted to 10 mL with TEE buffer to give a 1- μ g/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 μ L of a 2×10^{-7} M [³H]estradiol solution in TEE buffer, followed by 200- μ L aliquots of the competitor solutions, and the tubes were vortexed. After addition of 0.5 μ L 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 μ L 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- μ L 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.

17 β -[6,7-³H(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 [16 α ,17 α -d]Isoxazolidines

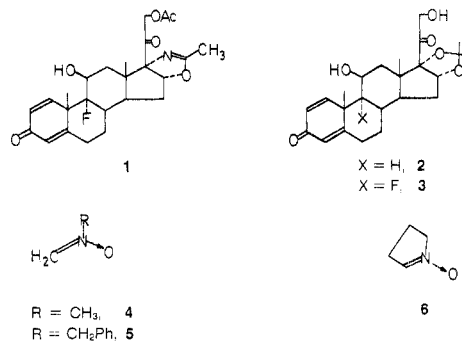
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1,3-Dipolar cycloaddition of *N*-methylnitrone, *N*-benzylnitrone, and pyrroline *N*-oxide to 1,4,16-pregnatriene-3,20-diones is described. In each case only [16 α ,17 α -d]isoxazolidines were isolated. The pentacyclic adducts **16**–**19** were active 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 [17 α ,16 α -d]-2'-methyloxazoline **1**² and the 16 α -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



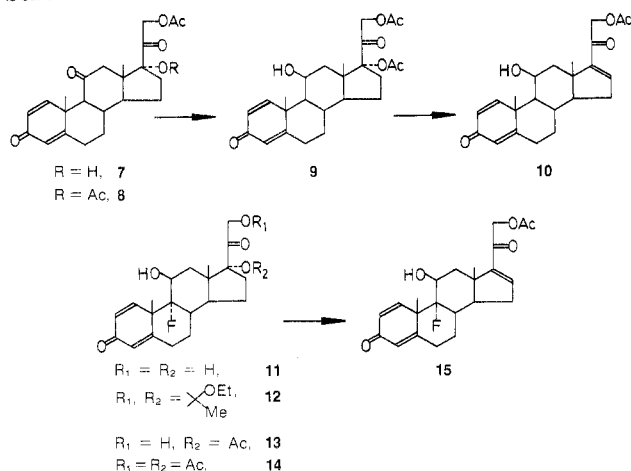
describe the preparation and topical antiinflammatory activity of a series of [16 α ,17 α -d]isoxazolidines. We chose

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Scheme I



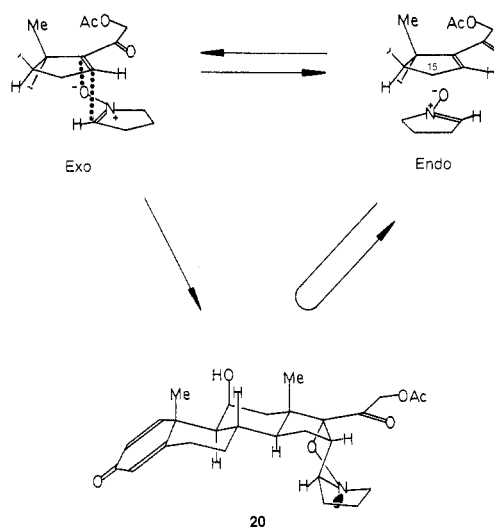
this particular heterocycle because it combines two structural features of high-potency, systemic corticosteroids (an oxygen function at C₁₇ and a one-carbon fragment at C₁₆) with a heterocyclic ring fused at C₁₆ and C₁₇, which is a feature of the topically active group of compounds exemplified by 1–3.

We envisaged that the desired [16 α ,17 α -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 Neuklis⁴ reported that the addition of C,N-diphenylnitron to 16-dehydropregnenolone acetate gave both the [16 α ,17 α -d]- and the [17 α ,16 α -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) [(CF₃CO)₂O, *p*-TSA, HOAc], followed by selective reduction⁶ (NaBH₄) of the 11-ketone, gave prednisolone 17,21-diacetate (9). Treatment of 9 with KOAc in DMF at 100 °C⁷ then afforded 10 (20% from 8). 9 α -Fluoroprednisolone 11 formed the corresponding 9 α -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*-methylnitron (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 ¹³C

Scheme II

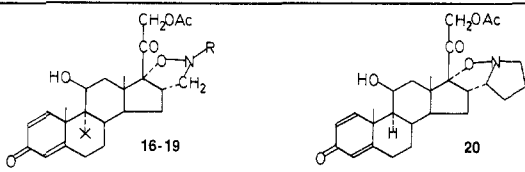


NMR spectra clearly showed that reaction had occurred at the 16,17 double bond, since the signals due to the cross-conjugated dienone system in ring A were still present. Compound 16 was the [16 α ,17 α -d]isoxazolidine because its ¹H NMR spectrum showed only one signal for a methine proton geminal to oxygen (the 11 α -H signal at 4.43 ppm). A spectrum of the regioisomeric [17 α ,16 α -d]isoxazolidine would have shown two such signals. This assignment was confirmed by ¹³C NMR spectroscopy; the spectrum of 16 showed C₁₇ at 99.97 ppm and C₁₆ 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 16 α ,17 α by analogy to the known direction of attack of other dipolarophiles to the 16,17 double bond.^{4,9} In addition, the ¹H and ¹³C NMR spectral data were clearly in accord with this assignment.

N-Benzylnitron and 10 gave the analogous isoxazolidine 17, and cycloaddition of *N*-methyl- and *N*-benzylnitrones to 15 produced the 9 α -fluoro analogues 18 and 19, respectively. Crystallization or chromatography isolated only the [16 α ,17 α -d]isoxazolidines in each of these three cases. Pyrroline *N*-oxide¹⁰ 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 C₃ stereochemistry, but inspection of models suggested that C₃ should be *R*. This assignment is explained in Scheme II. Aligning stereo-models of the dipole 6 and the dipolarophile 10 in parallel planes¹¹ 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 14 α hydrogen of 10 and one of the C₄ hy-

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Table I. Physical and Biological Data for Steroidal [16 α ,17 α -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 ₂ Ph	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 ₂ Ph	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 μ g was the highest dose tested.

drogens of 6. This destabilizing interaction should slow any cycloaddition, yielding the C₃, 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 modification¹⁴ 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 [16 α ,17 α -d]isoxazolidine series the introduction of the fluorine at C₉ 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 pyrrolidine N-oxide was, however, inactive at the highest dose tested; presumably, interaction of ring D and the substituents at 16 α and 17 α 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 ¹³C NMR spectra were obtained on Varian CFT-20 and XLFT-100 instruments, respectively, in CDCl₃ solution with Me₄Si as an internal standard. Silica gel preparative (2000 μ m) and analytical (250 μ m) 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).

11 β ,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-H₂O (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 Me₂CO/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 NaBH₄ (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 Me₂CO/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 CHCl₃. The organic extract was washed with water and dried (anhydrous Na₂SO₄), and the solvent was evaporated to give crude 10 (11 g), which was chromatographed on a column of silica gel (650 g). Elution with CHCl₃/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).

9 α -Fluoro-11 β ,21-dihydroxy-1,4,16-pregnatriene-3,20-dione 21-Acetate (15). 9 α -Fluoroprednisolone (11; 17 g) and *p*-TSA-H₂O (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 H₂O and dried (anhydrous Na₂SO₄), 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 H₂O, dried (anhydrous Na₂SO₄), 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 H₂O (1.6 L). The product was extracted into EtOAc, and the organic extract was washed with H₂O, dried (anhydrous Na₂SO₄), and concentrated to an oil (15.1 g). Addition of Et₂O 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 H₂O, and dried in vacuo to give crude 15 (8.5 g). This product was taken up in a minimum of CHCl₃ and filtered through a column of neutral alumina (30 g). The CHCl₃ eluate was concentrated to dryness (6.8 g) and then crystallized from Me₂CO/hexane to give 15 (2.6 g): mp 211-215 °C; $[\alpha]_D^{25} +128^\circ$ (DMF); UV λ_{\max} (MeOH) 238 nm (ϵ 24 200); ¹H NMR (Me₂SO-*d*₆) δ 1.17 (C₁₃ CH₃, s), 1.52 (C₁₀ CH₃, s), 2.08 (OCOCH₃, s), 4.02 (11-H, m), 4.84 and 5.12 (C₂₁ H's, d, *J* = 18 Hz), 6.00 (C₄ H, s), 6.10 (C₂ H, dd, *J* = 10 and 2 Hz), 6.96 (C₁₆ H, m), 7.30 (C₁ H, d, *J* = 10 Hz). Anal. (C₂₅H₂₇O₅F) C, H, F.

Steroid 2'-Methyl-Substituted [16 α ,17 α -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 H₂O, and the product was extracted into CHCl₃. The organic extract was washed with water, dried (anhydrous Na₂SO₄), and concentrated to an oil (1.48 g), which was chromatographed on a column of silica gel (150 g). Elution with EtOAc/CHCl₃ (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 (CDCl₃) δ 0.99 (C₁₃ CH₃, s), 1.46 (C₁₀ CH₃, s), 2.17 (OCOCH₃, s), 2.62 (N-CH₃, s), 3.46 (3'-H's, m, *W*_{1/2} = 10 Hz), 4.47 (11-H, m), 4.77 and 5.05 (C₂₁ H's, d, *J* = 18 Hz), 6.00 (C₄ H, s), 6.25 (C₂ H, dd, *J* = 10 and 2 Hz), 7.27 (C₁ H, d, *J* = 10 Hz); ¹³C NMR (CDCl₃) δ 17.0 (q, C₁₈), 20.5 (q, C₁₉), 21.1 (q, COCH₃), 30.6 (d, C₃), 31.0 (C₆, C₁₅), 34.1 (t, C₇), 40.8 (t, C₁₂), 44.1 (s, C₁₀), 44.4 (q, NCH₃), 45.2 (s, C₁₃), 48.1 (d, C₁₆), 50.8 (d, C₁₄), 55.5 (d, C₉), 66.3 (t, NCH₂), 67.5 (t, C₂₁), 69.9 (d, C₁₁), 100.0 (s, C₁₇), 122.5 (d, C₄), 127.8 (d, C₂), 156.3 (d, C₁), 169.9 (s, OCO), 170.6 (s, C₅), 186.5 (s, C₃), 205.1 (s, C₂₀). Anal. (C₂₅H₃₃O₆N) 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 (Me₂SO-*d*₆) δ 0.86 (C₁₃ CH₃, s), 1.48 (C₁₀ CH₃, s), 2.09 (OCOCH₃, s), 3.28 (N-CH₃, s), 3.40 (3'-CH₂, m), 4.12 (11 H, m), 4.86 (C₂₁ H's, s), 5.99 (C₄ H, s), 6.10 (C₂ H, dd, *J* = 10 and 2 Hz), 7.27 (C₁ H, d, *J* = 10 Hz); ¹³C NMR (CDCl₃) δ 16.5 (q, C₁₈), 20.5 (q, COCH₃), 21.1 (q, C₁₉), 27.7 (t, C₁₅), 31.1 (C₇, *J*_{CF} = 5 Hz), 33.5 (C₆), 33.5 (d, C₃, *J*_{CF} = 15 Hz), 37.1 (t, C₁₂), 44.0 (C₁₄, C₁₆, NCH₃), 48.17 (s, C₁₃), 48.4 (s, C₁₀, *J*_{CF} = 23 Hz), 66.2 (t, NCH₂), 67.6 (t, C₂₁), 71.7 (d, C₁₁, *J*_{CF} = 38 Hz), 99.8 (s, C₁₇), 100.5 (s, C₉, *J*_{CF} = 177 Hz), 125.0 (d, C₄), 129.7 (d, C₂), 152.5 (d, C₁), 166.3 (s, C₅), 170.7 (s, COCH₃), 186.7 (s, C₃), 205.2 (s, C₂₀). Anal. (C₂₅H₃₂O₆NF) C, H, N, F.

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Steroidal 2'-Benzyl-Substituted [16 α ,17 α -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 H₂O, and the precipitate was filtered off, washed with water, and air-dried. This product was chromatographed on silica gel (70 g), eluting with CHCl₃/EtOAc (3:1) to give 17 (0.34 g), which was crystallized from Me₂CO/hexane to give pure 17 (0.191 g): ¹H NMR (Me₂SO-*d*₆) δ 0.82 (C₁₀ CH₃, s), 1.38 (C₁₃ CH₃, s), 2.06 (OCOCH₃, s), 3.37 (3'-H's, m), 3.84 (CH₂Ph, d, *J* = 4 Hz), 4.28 (11-H, m), 4.50 (C₂₁ H's, s), 5.91 (C₄ H, s), 6.13 (C₂ H, dd, *J* = 10 and 2 Hz), 7.30 (C₁ H and phenyl H's, m); ¹³C NMR (CDCl₃) δ 17.0 (q, C₁₈), 20.5 (q, C₁₉), 21.1 (q, COCH₃), 30.6 (d, C₈), 32.0 (C₆, C₁₅), 34.2 (t, C₇), 40.7 (t, C₁₂), 44.2 (s, C₁₀), 45.3 (s, C₁₃), 47.4 (d, C₁₆), 50.8 (d, C₁₄), 55.6 (d, C₉), 61.9 (t, NCH₂Ph), 64.1 (t, NCH₂), 67.6 (t, C₂₁), 69.8 (d, C₁₁), 99.6 (s, C₁₇), 122.5 (d, C₄), 156.4 (d, C₁), 170.0 (s, COCH₃), 170.5 (s, C₅), 186.5 (s, C₃), 205.0 (s, C₂₀). Anal. (C₃₁H₃₇O₆N) 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 (Me₂SO-*d*₆) δ 0.84 (C₁₃ CH₃, s), 1.50 (C₁₀ CH₃, s), 2.06 (OCOCH₃, s), 3.38 (3'-H's, m), 3.95 (CH₂Ph, d, *J* = 4 Hz), 4.14 (11-H, m), 4.52 (C₂₁ H's, s), 6.00 (C₄ H, s), 6.10 (C₂ H, dd, *J* = 10

and 2 Hz), 7.28 (C₁ H and phenyl H's). Anal. (C₃₁H₃₆O₆NF) C, H, N, F.

Steroidal 2',3'-Trimethylene-Substituted [16 α ,17 α -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 H₂O, dried (anhydrous Na₂SO₄), and concentrated under reduced pressure to an oil (0.619 g). Separation of this product by preparative thin-layer chromatography (development solvent CHCl₃) gave pure 20 (0.399 g). A portion was crystallized from CHCl₃/Me₂CO to give 20: mp 209-212 °C; ¹H NMR (CDCl₃) δ 0.94 (C₁₃ CH₃, s), 1.44 (C₁₀ CH₃, s), 2.16 (OCOCH₃, s), 3.1-3.6 (3'-H's and pyrrolidine H's, m), 4.50 (11-H, m), 4.67 and 4.96 (C₂₁ H's, d, *J* = 18 Hz), 5.99 (C₄ H, s), 6.22 (C₂ H, dd, *J* = 10 and 2 Hz), 7.22 (C₁ H, d, *J* = 10 Hz). Anal. (C₂₇H₃₄O₆N) 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 nature³ were assigned to the glycosides by means of gas chromatography,⁴ mass spectrometry,⁵ and ¹³C nuclear magnetic resonance (¹³C NMR) determination.⁶ As a potent tool, ¹³C NMR was effectively applied to the configurational and conformational analyses. The ¹³C 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 corresponding set of ring carbons in alkyl D-fructofuranosides, as was demonstrated in D-fructose. As a result of ¹³C NMR spectral studies,

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