giving 1 (40.3 g, 66%), mp 180–182°. Recrystallization from EtOH gave pure 1, mp 184–185°.

N-(2-Acetoxyethyl)-2-[1-(2-acetoxyethyl)-5-nitroimidazol-2-yl]-nitrone (20). A solution of 18 (20 g, 0.07 mol), Ac_2O (12 ml), pyridine (11 ml), and THF (200 ml) was refluxed for 4 hr and evaporated. The residue was recrystallized from C_6H_6 (150 ml) to give 20 (13.8 g, 59%), mp 93–96°.

Biological Test Procedures. The *in vitro* testing of the compounds was carried out using the paper disk method. In this method a standard 12.7-mm diameter sterile filter paper disk impregnated with 0.1 ml of the indicated concentration of the compound was placed on a trypticase soy agar plate seeded with the test organism. The seeded plates were incubated for 24 hr at 37° and then observed for zones of inhibition of bacterial growth around the disks.

In vivo testing was carried out in CF_1, ♂, 18–20-g mice. The compounds were administered subcutaneously or orally. Two doses of compound were given on both day 1 and 2, with the bacterial challenge given intraperitoneally between doses on day 1. Doses protecting 50% of the animals were calculated by the method of Reed and Muench[8] as milligrams per kilogram based on individual doses and not total compound given.

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7-Acetylthioetiojervanes, New Antialdosterone Agents

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Several 12α-etiojervane derivatives have been found to be potent antialdosterone agents when administered parenterally.† In an attempt to increase the oral efficacy of these compounds, structural modifications were made paralleling those successfully employed for a similar purpose in the steroidal spirolactones, a class of well-known antialdosterone agents.[2,3] The particular molecular changes reported in this communication involve the introduction of the acetylthio group at the 7α position of the etiojervane molecule.

Direct chloranil oxidation of the unsaturated ketone **1d** led to mixtures containing moderate amounts of the desired dienone **2d** as evidenced by the uv spectrum of the crude product. Purification of this material was hampered by partial decomposition of the dienone **2d** on chromatography. The partially purified material was saponified at room temperature to yield the desired alcohol **2a**. The structure of this dienone was unambiguously shown by its spectral profile.

The lack of stability of the unsaturated ketone **1** was also seen when acid-catalyzed ketal[1a] or enol ether formation was attempted. One example of this instability was the intractable mixture produced by room temperature treatment of the unsaturated ketone **1a** with trimethyl orthoformate and acid. This instability presumably reflects the steric strain imposed by the cis-fused C/D hydrindan system. However, enol ether formation was successfully achieved by use of dimethoxypropane. Subsequent mild oxidation with dichlorodicyanoquinone (DCDCQ) gave the desired dienone in an overall yield of 40% from the starting unsaturated ketone **1a**.

The addition of thiolacetic acid was found to proceed readily at room temperature in contrast to the standard procedure which employs prolonged heating.[2] The product was a stable, crystalline product. Assignment of the 7α configuration to the acetylthio group was done in analogy to the spirolactone work, since no obvious steric factors would favor addition of the thiolacetic acid to the β face of the molecule. Corroboration of this configurational assignment was obtained from the nmr spectrum of this molecule and its comparison to the spectrum of the corresponding spirolactone.[4]

Derivatives with a 17α substituent were also prepared. The starting 4,6-diene **2e** could be obtained in 15% yield by direct crystallization of the product resulting from dehydrobromination of the 17α-acetoxy-2,4-dibromo derivative **6** (X = Br). This type of elimination has been previously noted with steroidal 2,4-dibromo 3-ketones.[5] The dienone **2e** could also be obtained by direct oxidation of the 3-keto-Δ^4 compound **1e** with DCDCQ but again this procedure gave only a low yield of product. Saponification of the 17α-acetate **2e** gave the dienone **2b**. Alternatively, the 17α-acetate **2b** was synthesized by room temperature DCDCQ oxidation of the enol ether **4b**. Thiolacetic addition proceeded smoothly to give the desired thiolacetate **5b** (or **5e**) from the 17α-hydroxy derivative **2b** (or its acetate **2e**) (Scheme I).

The 17-keto dienone **2c** was prepared from the 17α-hydroxy derivative **2b** by oxidation of the corresponding alcohol with chromic acid. To circumvent possible isomerization of the labile 13-methyl group,[6] the 7α-thiol acetate was prepared by oxidation of either the 17β- or the 17α-hydroxy derivative **5a** or **5b**, reactions which proceeded without complication to give the desired 17-keto derivative **5c**.

Sequential treatment of the 1,4-diene **3d** with *N*-bromosuccinimide and magnesium oxide or treatment of the 4,6-dienone **2a** with DCDCQ failed to give discernible amounts of the 1,4,6-trienone corresponding to **2**.

Biological Activity. The compounds were assayed in a 4-hr test in adrenalectomized rats treated with desoxycorticosterone acetate (DCA) and isotonic saline solution prior to the administration of the test compound.[7] The enhancement of potencies produced in the spirolactones by these chemical modifications were not observed in the etiojervanes (see Table I). In the present case the potent activity of the parent compound **1a** (450%) was decreased sharply by either introduction of the 6,7 double bond (50%, **2a**) or the 7-thioacetate (52%, **5a**). Of the compounds possessing parenteral activity, only **5a** was active orally but at a reduced (13%) potency.

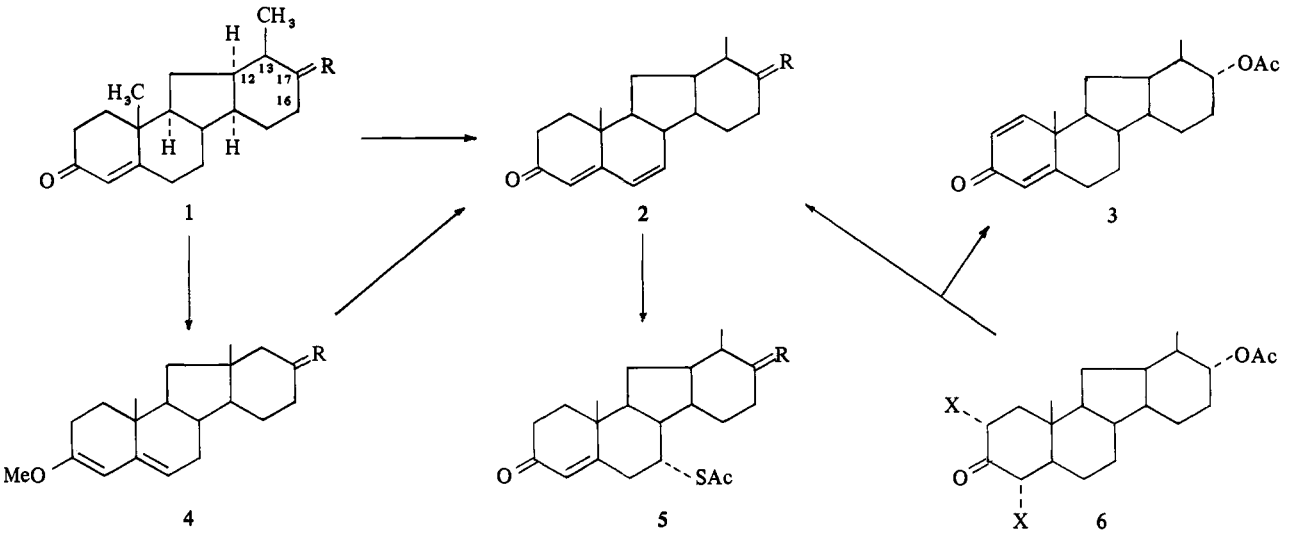
Experimental Section‡

3-Methoxy-12α-etiojerva-3,5-dien-17β-ol (4a) (Procedure A). *p*-Toluenesulfonic acid (50 mg) was added to a solution of 1.53 g of

†See ref 1a for the chemical synthesis of these compounds. The biological activities will be described in a forthcoming paper by L. M. Hofmann and W. F. Johns. See also ref 1b.

‡Optical rotations were determined in chloroform, infrared spectra in chloroform, ultraviolet spectra in methanol, and nmr spectra in deuteriochloroform. Nmr's are reported with tetramethylsilane as an internal standard ($\Delta\nu = 0$) as recorded on a Varian A-60 spectrometer. These data were supplied by Dr. J. W. Ahlberg and staff for which we thank them.

Scheme I



a, R = β-OH, H
b, R = α-OH, H
c, R = O
d, R = β-OAc, H
e, R = α-OAc, H

Table I. Antialdosterone Potencies[a]

No.	R 17β-OH (a)	17=O (c)	17α-OH (b)
2	50	I57	I
5	52	I	

[a]The figures given are the per cent of activity of 3-(3-oxo-17β-hydroxyandrost-4-en)-17α-ylpropionic acid lactone (SC-5233) in the standard Kagawa assay which employs adrenalectomized rats pretreated with DCA. The compounds were administered subcutaneously.

17β-hydroxy-12α-etiojerv-4-en-3-one (1a)[1,†] in 30 ml of dimethoxypropane (redistilled), 30 ml of DMF, and 0.6 ml of MeOH. The reaction ran for 5 hr at room temperature and for 1 hr at reflux. The cooled solution was then treated with 0.6 g of $NaHCO_3$. After 5 min, the mixture was poured into cold dilute aqueous KOH and extracted with CH_2Cl_2. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated. The resulting oil (1.75 g) failed to crystallize because of a polymeric (?) contaminant but exhibited acceptable spectral characteristics: uv 240 nm (ε 15,400); ir 3618, 1710 (w), 1660 cm^{-1} (polymer ?); nmr 58 (19-Me), 54 (?), 58, and 66 (18-Me), 314 and 319 Hz (C=CH). The use of trimethyl orthoformate gave the same enol ether contaminated with 3,3-dimethyl ketal and decomposition products.

17β-Hydroxy-12α-etiojerva-4,6-dien-3-one (2a). a. Enol Ether Oxidation (Procedure B). A solution of 1.7 g of the crude enol ether 4a (as prepared above) in 90 ml of 95% aqueous Me_2CO was treated with 1.05 mol equiv of DCDCQ at room temperature. After 20 min the solution was diluted with H_2O and extracted with C_6H_6. The extract was washed with cold aqueous KOH, H_2O, Na_2SO_3, and H_2O. The product, isolated as above, crystallized directly and was recrystallized from CH_2Cl_2–hexane to yield 0.58 g (40%) of the diene 2a: mp 175–179°; uv 286 nm (ε 23,700); ir 3618, 1650, 1605 cm^{-1}; nmr 60 and 67 (19-Me), 67 (18-Me), 340 (6-H), 375 Hz (m, J = 11 Hz). *Anal.* ($C_{19}H_{26}O_3$) C, H. Additional material could be isolated by a rapid chromatography of the material on silica gel, although some instability of the product was noted on this adsorbent.

b. Oxidation of the Unsaturated Ketone 1d. A solution of 1.15 g of the acetate 1d in 150 ml of *tert*-BuOH containing 1.1 mol equiv of chloranil (0.92 g) was boiled under an atmosphere of N_2 for 0.5 hr. The solvent was distilled (T <50°) and the residue was diluted with H_2O and extracted with CH_2Cl_2. The mixture was washed with dilute KOH three times, with H_2O, and twice with aqueous Na_2SO_3 and again with H_2O. Evaporation of the solvent gave 1.68 g of crude product; λ_{max} 243 nm (ε 7050), 285 (14,000) which was contaminated with a chlorinated material (found, 8.93% Cl). Chromatography of this material gave the product as an impure oil, λ_{max} 287 nm (ε 12,100), at 5% ethyl acetate–benzene.

The acetate 2d (95 mg; prepared from 1d with chloranil as described above) in 4 ml of MeOH and 0.7 ml of 10% aqueous KOH was allowed to stand at room temperature for 18 hr. The solution was diluted with H_2O and the product isolated by CH_2Cl_2 extraction. The crystalline product (27 mg) was identical with that obtained in part a.

7α-Acetylthio-17β-hydroxy-12α-etiojerv-4-en-3-one (5a). The dienone 2a (0.16 g) was added to 1 ml of AcSH. The mixture was homogeneous after 10 min. After 2.5 hr the acid was distilled (T <40°) and the residue was chromatographed quickly on 3 g of silica gel. Fractions eluted with 10% EtOAc–C_6H_6 crystallized and were recrystallized from Et_2O to yield 0.14 g of the thiolacetate 5a: mp 147–152°; uv 238 nm (ε 18,500); ir 3625, 1678 cm^{-1}; nmr 58 and 64 (18-Me), 70 (19-Me), 139 (SAc), 231 (m, 17-H), 252 (m, 7-H), 244 Hz (d, 4-H). *Anal.* ($C_{21}H_{30}O_3S$) C, H.

17α-Acetoxy-12α-etiojerva-4,6-dien-3-one (2e). a. Dibromination–Dehydrobromination. The acetate 6e (X = H; 16 g) in 220 ml of AcOH was treated with 133 ml of an 0.80 M solution of Br_2–AcOH (2.2 mol equiv) over a 45-min period. The pale yellow solution was diluted with H_2O and the resulting precipitate was collected and washed well with H_2O. Recrystallization of this material afforded 17.3 g of the impure bromide 6 (X = Br), essentially identical with the material described earlier.[1,†]

A solution of the crude dibromide (17 g) in 80 ml of DMF was added to a vigorously refluxing solution of 4.83 g of Li_2CO_3 and 0.14 g of LiCl in 240 ml of DMF. After 1 hr the solution was cooled and poured into H_2O. The resulting oily precipitate was separated by filtration, washed with H_2O, and dissolved in C_6H_6. This solution was washed with H_2O, dried ($MgSO_4$), and concentrated, yielding a noncrystalline product. Crystallization followed by fractional crystallization afforded, besides the 1,4-dienone 3, 3.6 g of the 4,6-dienone 2e ($\epsilon_{284\ nm}$ 19,200). Recrystallization of this material from Et_2O–hexane afforded 1.65 g (15%) of the pure dienone: mp 139–142°; ir 1665, 1730 cm^{-1}; uv 285 nm (ε 25,700); nmr 53 and 59 (18-Me), 67 (19-Me), 125 (OAc), 346 (4-H), 370 (6-H), and 377 Hz (7-H). *Anal.* ($C_{21}H_{28}O_2$) C, H. Chromatography of the mother liquors of this reaction afforded another 2.8 g of the crude 4,6-dienone, eluted at 20% EtOAc–C_6H_6.

b. Direct Oxidation of the Unsaturated Ketone 1e with DCDCQ. A solution of 225 mg of the acetate 1e and 162 mg of DCDCQ in 20 ml of dioxane was treated for 5 sec with a stream of anhydrous HCl. A precipitate formed within 2 min. After 15 min more, the mixture was filtered and the precipitate was rinsed with Et_2O. The filtrate was washed twice with aqueous Na_2SO_3 and then with aqueous KOH solution. The Et_2O layer afforded 215 mg of the crude dienone acetate, $\epsilon_{285\ nm}$ 10,400. Attempts to purify this material by chromatography effected considerable decomposition as indicated by the

decrease in the overall intensity of the uv maximum at 285 nm. A similar crude preparation containing the 4,6-dienone was obtained by boiling the compound in *tert*-BuOH with an equal weight of chloranil.

17α-Hydroxy-12α-etiojerva-4,6-dien-3-one (2b). a. Oxidation of the Enol Ether 4b. The unsaturated ketone 1b (1 g) was treated with dimethoxypropane according to procedure A and yielded an amorphous enol ether (1.0 g). This material was treated with DCDCQ in Me_2CO (procedure B), affording, by direct crystallization from Et_2O, 0.46 g of dienone 2b: mp 133–139°; ir 3680, 1660 cm^{-1}; uv 286 nm (ε 27,000); nmr 59 and 66 (18-Me), 66 (19-Me), 345 (4-H), 370 (6-H), 378 Hz (7-H). *Anal.* ($C_{19}H_{26}O_2$) C, H.

b. Saponification of the Acetate 2e. A solution of 0.20 g of the dienone 2e was stirred in 20 ml of *tert*-BuOH and 3 ml of 10% aqueous KOH for 18 hr. The solution was diluted with H_2O and extracted with Et_2O. The resulting product was chromatographed quickly and yielded, by elution with 20% EtOAc–C_6H_6, 75 mg of the dienone 2b, identical with that produced above.

12α-Etiojerva-4,6-diene-3,17-dione (2c). A solution of 0.11 g of the alcohol 2b in 10 ml of pyridine was oxidized with the Sarett reagent[8] prepared from 0.5 g of CrO_3 at room temperature. The product was isolated by ether extraction and was crystallized from aqueous Me_2CO to yield 35 mg of the diketone as a hemihydrate: mp 180–183°; ir 1720, 1750 cm^{-1}; uv 284 nm (ε 24,100); [α]D −33°. *Anal.* ($C_{19}H_{24}O_2 \cdot H_2O$) C, H.

7α-Thioacetyl-17α-acetoxy-12α-etiojerv-4-en-3-one (5e). The acetate 2d was allowed to stand in 1 ml of AcSH at room temperature for 24 hr. The solvent was removed under vacuum and the product was chromatographed quickly on a short bed of silica gel. The material eluted with 10% EtOAc–C_6H_6 crystallized from Et_2O and was recrystallized from Et_2O–hexane to yield 80 mg of the pure adduct 5e: mp 152–155°; ir 1730, 1700, 1670 cm^{-1}; uv 237 nm (ε 14,700); nmr 53 and 59 (18-Me), 71 (19-Me), 123 (OAc), 142 (SAc), 343 Hz (4-H). *Anal.* ($C_{23}H_{32}O_4S$) C, H.

7α-Acetylthio-17α-hydroxy-12α-etiojerv-4-en-3-one (5b). The dienone 2b (0.40 g) was allowed to stand in 1 ml of AcSH for 2 hr. The solvent was removed from Et_2O to give 0.25 g of the adduct 5b: mp 104–107°; ir 3680, 1710 cm^{-1}; uv 238 nm (ε 17,500); nmr 59 and 66 (18-Me), 71 (19-Me), 142 (SAc), 347 Hz (4-H). *Anal.* ($C_{21}H_{30}O_3S$) C, H; C: calcd, 69.58; found, 69.13; H: calcd, 8.34; found, 8.80.

7α-Acetylthio-12α-etiojerv-4-ene-3,17-dione (5c). A solution of 0.20 g of the alcohol 5b in 5 ml of Me_2CO was oxidized with 0.2 ml of 4 *N* CrO_3 (Jones reagent)[9] at 5°. After 10 min the solution was diluted with H_2O and the resulting precipitate was separated. Recrystallization from aqueous Me_2CO gave 120 mg of the ketone 5c: ir 1710, 1692, 1679 cm^{-1}; uv 238 nm (ε 16,100); nmr 57 and 63 (18-Me), 68 (19-Me), 142 (SAc), 347 Hz (4-H). *Anal.* ($C_{21}H_{28}O_3S$) C, H. Oxidation of the 17β-alcohol 5a proceeded in an analogous fashion to provide the same ketone 5c.

Allylic Bromination of 17β-Acetoxy-12α-etiojerva-1,4-dien-3-one (3d). The 1,4-diene 3d was treated with an equal weight of NBS in refluxing CCl_4. The resulting bromide on dehydrobromination with MgO gave dark mixtures containing no visible 1,4,6-trienone by spectral analysis (uv, nmr). An attempt to prepare the 1,4,6-trienone from the 4,6-diene 2a by use of DCDCQ in refluxing C_6H_6 for 20 hr afforded a dark oil containing some starting material but no discernible trienone (uv, nmr analysis).

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Synthesis of Nucleoside 5′-Carbamates†

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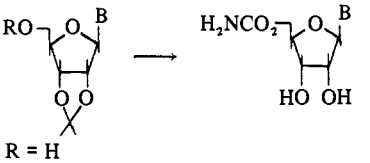
One of the difficulties encountered in the use of nucleosides in chemotherapy is that the nucleoside often requires conversion *in vivo* to a nucleotide for activation. Drug resistance then develops when the cells fail to perform this conversion.[1,2] Direct use of the nucleotide generally fails, presumably due to either enzymatic cleavage or failure of the highly charged molecules to cross the cell membrane. Several workers have tried, with varying degrees of success, to overcome this problem by substituting polar but nonionized groups for the 5′-phosphate.[1-6] Baker and coworkers, working *in vitro* with isolated enzyme systems, found evidence for simulation of phosphate by *O*-carbamate in a derivative of 6-mercaptopurine[3] but were unable to detect it with two other nucleoside-derived carbamates.[5] Because of the above theoretical considerations and Baker's partial success *in vitro*, as well as the occurrence of the *O*-carbamyl moiety in a variety of antitumor substances (*e.g.*, mitomycin,[7] bleomycin,[8] the acetylenic diarylcarbinol carbamates[9,10]), we were stimulated to prepare 5′-*O*-carbamyl nucleosides for *in vivo* antineoplastic evaluation as part of our continuing studies in the area of fraudulent nucleosides.

The nucleoside 5′-*O*-carbamates were prepared by the route shown in Scheme I, essentially that of Baker, *et al.*[5]

Scheme I



a, R = H
b, R = CO_2Ph
c, R = $CONH_2$

Yields in the final step (removal of the isopropylidine blocking group) were substantially improved by the use of 90% trifluoroacetic acid.[11] Intermediates in the preparation of the 6-methylthiopurine nucleoside 7 could not be isolated pure and would not crystallize. An attempt to apply this sequence to 2′,3′-isopropylidene adenosine resulted in a mixture of products at the phenylchloroformylation stage.

Intermediate 4 (see Table I) was not obtained pure and therefore was not submitted for antitumor screening. The other compounds were inactive against leukemia L-1210 in mice treated on days 1–5 with doses of 200 mg/kg/day in standard NCI assays.‡ In addition, compounds 1, 2, 5, 6, and 8 were without cytotoxicity against KB cells in tissue culture at 100 μg/ml.

Experimental Section

General Procedure.§ **6-Methylthio-9-(2′,3′-*O*-isopropylidine-β-D-ribofuranosyl)purine (3).** A mixture of 6-methylthio-9-(β,D-ribofuranosyl)purine (2.0 g, 6.7 mmol), acetone (100 ml), anhydrous copper sulfate (4.0 g), and concentrated sulfuric acid (4 drops) was stirred in a stoppered flask for 4 days. The suspension was filtered and the

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‡Screening was performed under the auspices of DR&D according to the protocols described in ref. 12.

§See Table I for additional details.