tions from ethanol when heated softened at 217° and melted with gas evolution at 221°: ir (Nujol) 3266 (NH_2), 1681 (CO), 1600 cm^{-1} (C=N).

Anal. Calcd for $C_{15}H_{18}N_4O_2$: C, 62.94; H, 6.29; N, 19.58. Found: C, 63.03; H, 6.04; N, 19.81.

Thermolysis of 5. Compound **5** (1.09 g) was heated at 220–230° for 15 min and the resulting solid was treated with water. Filtration gave 0.47 g of a solid which was extracted with chloroform and recrystallized twice from ethanol, mp 372–384°. The ir spectrum was similar to that of the compound isolated in the reaction of the diamine **1** with *trans*-di-*p*-toluylethylene in ethanol.

Condensation of Diamine 1 with 1,2-Di-*p*-toluylethane. The diamine **1** (0.85 g) was heated at reflux with 1,2-di-*p*-toluylethane (1.33 g) in absolute ethanol (50 ml) under nitrogen for 31 hr. The solution upon filtration gave 1.50 g of the pyrrol **8** which after recrystallization successively from ethyl acetate and methanol melted at 303–309° dec; ir (Nujol) 3333 (NH), 1701 (CO), 1600 cm^{-1} (C=C); NMR (Me_2SO-d_6) δ 2.23 [s, 6, $(CH_3)_2$], 3.13 (s, 3, NCH_3), 3.18 (s, 3, NCH_3), 6.26 (broad s, 2, NH_2), 6.39 (s, 2, =CH), 7.03 (d, 4, meta ArH, J = 8 Hz), 7.30 (d, 4, ortho ArH, J = 8 Hz). Upon the addition of D_2O the peak at δ 6.26 disappeared, *m/e* 400.

Anal. Calcd for $C_{24}H_{24}N_4O_2$: C, 72.0; H, 6.0; N, 14.0. Found: C, 71.86; H, 6.00; N, 13.82.

Registry No.—**1**, 5440-00-6; **2** (R = CH_3), 57196-68-6; **2** (R = C_6H_5), 57196-69-7; **3** (R = CH_3), 830-65-9; **3** (R = C_6H_5), 961-45-5; **3** (R = *p*-$CH_3C_6H_4$), 57196-70-0; **4**, 51445-58-0; **5**, 57196-71-1; **6**, 57196-72-2; **7**, 57196-73-3; **8**, 57196-74-4; 5-nitroso-4-amino-1,3-dimethyluracil, 6632-68-4; acetylacetone, 123-54-6; 5-acetylamino-4-amino-1,3-dimethyluracil, 10184-41-5; dibenzoylmethane, 120-46-7; *trans*-di-*p*-toluylethylene, 17342-09-5; 2,5-di-*p*-tolylfuran, 57196-75-5; *p*-methylacetophenone, 122-00-9; 1,2-di-*p*-toluylethane, 13145-56-7.

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Synthesis of β,γ-Acetylenic 3-Oxo Steroids of the 5,10-Seco Series[1]

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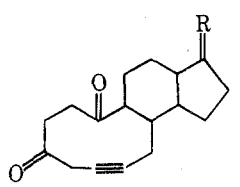
Received July 8, 1975

The synthesis is reported of 5,10-seco-19-norcholest-5-yne-3,10-dione (**1a**) and its estryne and 19-norpregnyne analogues (**1b** and **1c**, respectively). The key $\Delta^{5(10)}$-6-oxo intermediates (**3a–c**), prepared by direct chromium trioxide–pyridine oxidation of the corresponding Δ^5-19-hydroxy steroids (**2a–c**), were converted to the 5β,10β-oxido-6-oxo steroids (**4a–c**) using alkaline hydrogen peroxide or *m*-chloroperbenzoic acid. Compounds **4a–c** gave, after Tanabe–Eschenmoser fragmentation, the 3β-acetoxy-5,10-seco-5-yne derivatives (**5a–c**), which in turn yielded **1a–c**, after hydrolysis of the acetoxy groups followed by Jones oxidation. The 5β,10β configuration in the 5,10-oxido-6-oxo steroids was assigned on the basis of CD and of chemical evidence. Another route to the 5β,10β-oxido-6-ketone grouping was found in treatment of the $\Delta^{5(10)}$-6β-acetoxy compound **10** with Jones reagent to give directly the 5β,10β-oxido-6-ketone **4b**.

Studies concerned with the design and synthesis of specific enzyme-generated inhibitors of the Δ^5-3-keto steroid isomerase of *P. testosteroni* required the synthesis of 5,10-seco acetylenic steroids with the structures **1a–c.**



1a, R = β-C_8H_{17}, α-H
b, R = O
c, R = β-CH_3CO, α-H

These compounds were designed as substrates for Δ^5-3-keto steroid isomerase[2] with the hope that the enzyme, through its normal mode of action,[2,3] would convert the β,γ-acetylenic ketone system to the conjugated allenic ketone grouping. Thus, the enzyme converts Δ^5-3-oxo steroids to the corresponding Δ^4-3-oxo steroids by removing the 4β proton which is transferred intramolecularly to the 6β position, most plausibly via an enol intermediate. If compounds such as **1** proved to be substrates for the enzyme, the same process should generate the reactive $\Delta^{4,5}$-dien-3-one system. It was hoped that the latter would then react with a nucleophilic amino acid residue at or near the active site.

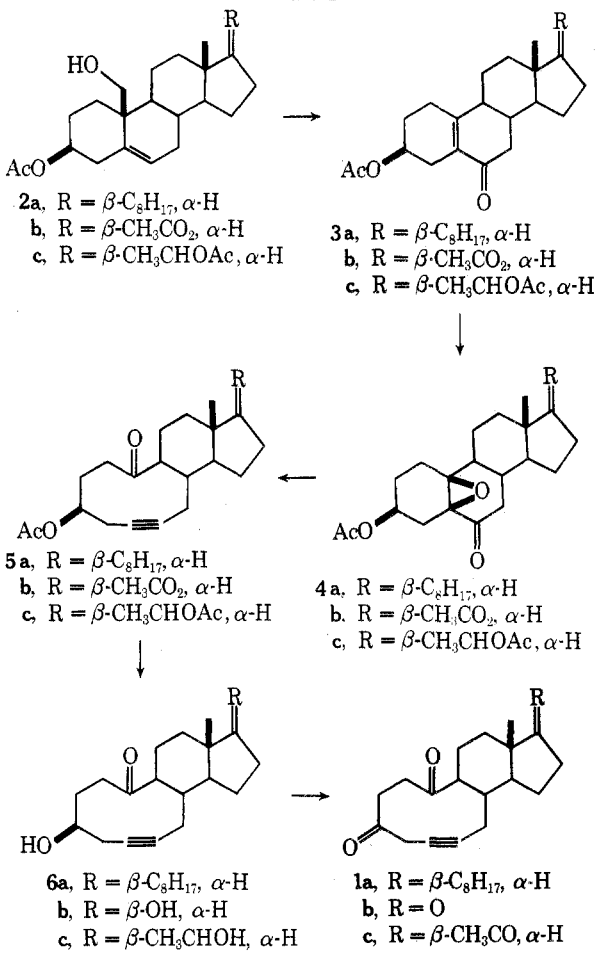
Examination of models suggested that these 5,10-seco steroids, with the ten-membered ring partly rigidified by the constraints of the acetylenic and carbonyl groupings, as well as by the ring junction at C-8 and C-9, might approximate conformationally to the natural tetracyclic Δ^5-3-oxo steroid system. In the event, seco steroids **1b** and **1c** indeed proved[4] to be excellent substrates for, and potent irreversible inhibitors of, the enzyme.

We report here the synthetic routes leading to compounds **1a–c**, as illustrated in Chart I, initially carried out in the cholestane series for calibration purposes.

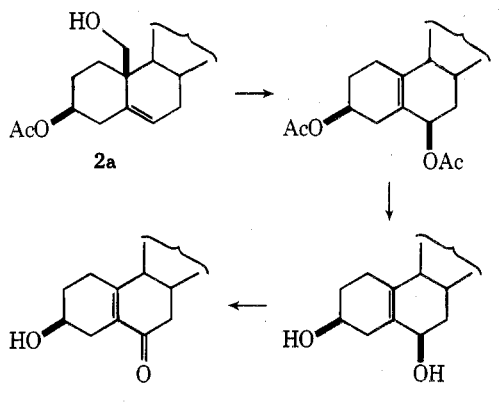
The critical part of the route involved generation of the key $\Delta^{5(10)}$-6-oxo intermediate (**3a**) with subsequent fragmentation of the derived 5β,10β-oxido-6-ketone (**4a**) to **5a** by the Tanabe–Eschenmoser[5,6] procedure. It was necessary to accumulate large quantities of the $\Delta^{5(10)}$-6-ketone (**3a**) and we first tried the known procedure[7] shown below.

This involved lead tetraacetate induced fragmentation of 3β-acetoxy-19-hydroxycholest-5-ene (**2a**) followed by cleavage of the acetoxy groups (lithium aluminum hydride) and selective oxidation at C-6 with manganese dioxide. In

Chart I



our hands this was not an entirely satisfactory process on the large scale, primarily owing to separation problems after the lithium aluminum hydride reaction.

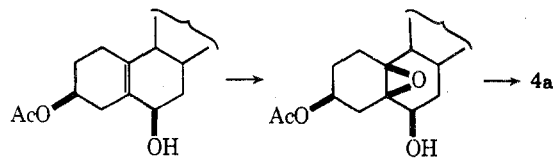


During our search for other ways to prepare the $\Delta^{5(10)}$-6-ketone **3a**, we were fortunate to have our attention directed[8] to an interesting and practical method described[9] in the patent literature. The method involves direct conversion of Δ^5-19-hydroxy steroids to $\Delta^{5(10)}$-6-ketones using chromium trioxide–pyridine at 25°.

Indeed, the $\Delta^{5(10)}$-6-ketone **3a** could be prepared conveniently in 40% yield on the large scale by this procedure (4 days at ambient temperature followed by chromatography on silica gel). Epoxidation of **3a** to give the oxido ketone **4a** was carried out using alkaline hydrogen peroxide, followed by reacetylation at C-3 for easier isolation and characterization.

The 5β,10β configuration for **4a** was inferred from the negative CD curve,[10] as well as from the following evidence.

The known 3β-acetoxy-$\Delta^{5(10)}$-6β-ol (**7**) of established[11] stereochemistry was converted quantitatively by *m*-chloroperbenzoic acid to the 5,10-oxido compound (**8**). The well-established[12] directive effect of the hydroxyl group in the peracid epoxidation of allylic alcohols argues most strongly for the 5β,10β configuration of the epoxide grouping in **8**. Oxidation of **8** with Jones reagent then gave in quantitative yield the oxido ketone **4a**, identical in all respects with **4a** which had been prepared by H_2O_2–NaOH epoxidation of the conjugated ketone **3a** followed by reacetylation at C-3.



The 5β,10β-oxido-6-ketone **4a** was then fragmented to give **5a** by the Tanabe–Eschenmoser reaction[5,6] (*p*-toluenesulfonyl hydrazide in acetic acid–chloroform at room temperature). Spectroscopic and analytical data were consistent with structure **5a** while positive evidence for the acetylenic grouping came from the Raman spectrum,[13] which showed absorption at 2230 cm^{-1}. In addition, catalytic hydrogenation of **5a** using Adams catalyst gave the tetrahydro derivative.

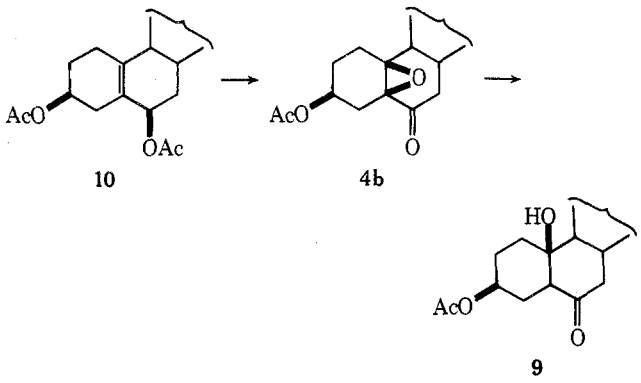
The 3β-acetoxy-5,10-seco steroid **5a** was now hydrolyzed to the 3β-ol **6a**, and oxidation with Jones reagent then afforded, in ca. 50% yield, the final product **1a**, which gave appropriate spectroscopic and analytical data.

The marked lack of reactivity of the C-10 carbonyl group in **5a** is noteworthy. Attempts to prepare the *p*-toluenesulfonylhydrazone failed, even under forcing conditions (e.g., *p*-toluenesulfonyl hydrazide and *p*-toluenesulfonic acid in sulfolane–dimethylformamide at 100°). Attempts to generate the oxime, using conditions (hydroxylamine–pyridine, reflux) suitable[14] for hindered 11-oxo steroids also failed. Furthermore, no reduction of the 10-ketone was observed under forcing Wolff–Kishner conditions, or by the use of sodium borohydride or lithium aluminum hydride. This lack of reactivity might be due in part to electronic interaction between the acetylene and 10-carbonyl functions. However, this is unlikely to be a major factor, as the tetrahydro compound obtained by catalytic hydrogenation of the acetylenic compound **5a** also proved to be inert to hydrazone-forming reagents. The formation of an intermediate complex would result in severe transannular interactions in the ten-membered ring, and this effect may play a critical role.

Turning now to the 5,10-secoestryne **1b** and 5,10-seco-19-norpregnyne (**1c**), we applied the synthetic sequence of Chart I as outlined above for the cholestane series. During these studies we observed that the 5β,10β-oxido ketone system (e.g., **4b**) can be conveniently prepared by treatment of the corresponding $\Delta^{5(10)}$-6-ketone with *m*-chloroperbenzoic acid in benzene under reflux. The undesired Baeyer–Villiger reaction did not compete to a major extent, and this procedure gave the crystalline oxido ketone **4b** directly in 67% yield without the reacetylation required after the H_2O_2–NaOH procedure.

The 5β,10β configuration for the oxido compounds **4b** and **4c** was inferred from their negative CD curves, as well as from analogy with the cholestane series. In addition, chromous acetate[15] reduction of **4b** gave the 10β-hydroxy-6-ketone **9**, which showed a negative CD spectrum as expected for a 6-oxo steroid of the 5α,10β series.

During a search for other paths to the 5β,10β-oxido ketone system, the interesting observation was made that Jones oxidation of the $\Delta^{5(10)}$-3β,6β,17β-triacetate[7] **10** led



directly within 10 min at 25° to the 5β,10β-oxido-6-ketone **4b.** Compound **4b** was identified by comparison with authentic samples prepared by the methods outlined above. The yield was only 20%, however, and the other reaction products have not yet been identified. The conjugated ketone **3b** is stable to these oxidation conditions, and further study of this reaction is planned.

Returning now to the synthesis of **1b** and **1c,** the final products **1b** and **1c** were obtained by fragmentation of oxido ketones **4b** and **4c,** followed by hydrolysis of the acetate groupings and oxidation with Jones reagent, as for the cholestane series.

We note, also, that hydrolysis of the acetoxy groups in **5a–c** with base does not cause epimerization at C-9. Thus, reacetylation of the hydrolyzed products **6a–c** generates, in quantitative yield, unchanged **5a–c** as evidenced by spectroscopic, chromatographic, and CD measurements.

Experimental Section

Melting points were determined on a Kofler hot stage and are uncorrected. NMR spectra were determined on Varian HA-100 or Perkin-Elmer R-12B spectrometers for $CDCl_3$ solutions, unless otherwise stated, with Me_4Si as internal standard. Chemical shifts are expressed as δ values (Me_4Si 0) with signal multiplicities shown as s, singlet; d, doublet; t, triplet; m, multiplet. Infrared spectra were obtained on Perkin-Elmer 137 or 521 spectrometers (in $CHCl_3$ solution unless otherwise stated). Ultraviolet spectra were measured on a Cary 15 spectrophotometer. Mass spectra were determined on CEC-21-110 or Du Pont 21-491 spectrometers. Circular dichroism measurements were made using a Cary 60 instrument and are expressed as molar ellipticities. Elemental analyses were performed by Microanalysis Inc., Wilmington, Del. All chromatographic separations were performed on Woelm dry column silica gel or alumina. Analytical thin layer plates (0.25 mm) were obtained from Analtech, Inc., Newark, Del. High-pressure liquid chromatographic separations were performed on a Waters Associates Model 6000 instrument equipped with a Model 660 solvent programmer.

3β-Acetoxy-19-norcholest-5(10)-en-6-one (3a). 3β-Acetoxy-19-hydroxycholest-5-ene (15.8 g, 0.036 mol) was dissolved in pyridine (100 ml) and stirred with chromium trioxide–pyridine complex (0.30 mol) at ambient temperature for 4 days. The mixture was diluted with EtOEt (1.5 l.) and filtered. The ethereal phase was washed successively with 5% NaOH and H_2O. The residue obtained by concentration in vacuo was chromatographed on silica gel by elution with 6% EtOAc–$CHCl_3$. Early fractions contained several nonpolar compounds followed by **3a** (5.7 g, 0.0133 mol, 36%): mp 122–123° (from MeOH) (reported[8] mp 124–125°); mass spectrum *m/e* 428 (M^+), 400, 382, 368; λ_{max} (MeOH) 246 nm (ε 11430); [θ] (MeOH) +2530° (329 nm); ν_{max} (KBr) 1725 (ester C=O), 1660 (C=O), 1625 cm^{-1} (C=C); NMR (C_6D_6) 4.50 (m, 1, CHOAc), 2.46 (m, 2, $COCH_2$), 2.00 (s, 3, CH_3CO), 0.85 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{28}H_{44}O_3$: C, 78.45; H, 10.35. Found: C, 78.43; H, 10.15

3β-Acetoxy-5β,10β-oxido-19-norcholestan-6-one (4a). 3β-Acetoxy-19-norcholest-5(10)-en-6-one (**3a,** 235 mg, 0.00055 mol) was dissolved in MeOH–$CHCl_3$ (15 ml:3 ml). At ambient temperature a mixture of 30% H_2O_2 (1 ml) and 5 *N* NaOH (1 ml) was added. After stirring for 5 hr the reaction mixture was diluted with aqueous NaCl (saturated) and extracted with $CHCl_3$. Drying (Na_2SO_4) and removal of solvent in vacuo left a solid which was acetylated with acetic anhydride–pyridine (18 hr, room temperature). After work-up in the usual manner the residue was crystallized from MeOH to give **4a** as needles (150 mg, 0.00033 mol, 58%): mp 173–174°; mass spectrum *m/e* 444 (M^+), 384, 368, 356, 340; ν_{max} 1735 (ester C=O), 1705 cm^{-1} (C=O); [θ] (CH_3OH) −8894° (307.5 nm); NMR 4.62 (m, 1, CHOAc), 2.02 (s, 3, CH_3CO), 0.70 ppm (s, 3, 18-CH_3)

Anal. Calcd for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.48; H, 9.79.

3β-Acetoxy-5β,10β-oxido-19-norcholestan-6-one (4a) from 3β-Acetoxy-6β-hydroxy-19-norcholest-5(10)-ene (7). A solution of the $\Delta^{5(10)}$-6β-ol[11] **7** (54 mg, 0.00013 mol) and 85% *m*-chloroperbenzoic acid (54 mg, 0.00027 mol) in $CHCl_3$ (8 ml) was left at ambient temperature for 18 hr. Water was then added, and the $CHCl_3$ phase was washed successively with 10% aqueous Na_2SO_3 solution, 10% $NaHCO_3$ solution, and water, and then was dried (Na_2SO_4) and evaporated. The residue was crystallized from acetone to give **8,** mp 140–141°, mass spectrum *m/e* 428 (M − 18), 386, 368.

Oxidation of the above product with Jones reagent in acetone at 5° for 15 min gave crude **4a** as a crystalline product. This material was homogenous by TLC, had mp 173–174°, and was identical in all respects (ir, NMR, CD, MS) with a sample of **4a,** prepared by H_2O_2–NaOH epoxidation of the conjugated ketone **3a** followed by reacetylation at C-3.

3β-Acetoxy-5,10-seco-19-norcholest-5-yn-10-one (5a). 3β-Acetoxy-5,10-oxidocholestan-6-one (**4a,** 666 mg, 0.0015 mol) and *p*-toluenesulfonyl hydrazide (333 mg, 0.0018 mol) were dissolved in 1:1 $CHCl_3$–AcOH (50 ml). After stirring for 5 hr at ambient temperature the reaction mixture was diluted with water and $CHCl_3$. The organic phase was washed with water and 5% $NaHCO_3$. Removal of the dried (Na_2SO_4) solvent in vacuo gave an oil which crystallized from MeOH, giving **5a** as plates (471 mg, 0.0011 mol, 73%): mp 105–106°; mass spectrum *m/e* 428 (M^+), 386, 368, 350; ν_{max} 1730 (ester C=O), 1705 cm^{-1} (C=O); [θ] (CH_3OH) −2805° (283 nm); NMR 4.80 (m, 1, CHOAc), 2.02 (s, 3, CH_3CO), 0.76 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{28}H_{44}O_3$: C, 78.45; H, 10.35; 0, 11.20. Found: C, 78.27; H, 10.35; 0, 11.20

3β-Hydroxy-5,10-seco-19-norcholest-5-yn-10-one (6a). The acetate **5a** (35 mg, 0.00008 mol) was dissolved in MeOH (7 ml) and stirred with anhydrous K_2CO_3 (150 mg) for 2 hr. Filtration and evaporation of the solvent left a solid which was crystallized from hexane to give **6a** as needles (30 mg, 0.000078 mol, 97%): mp 153–154°; mass spectrum *m/e* 386 (M^+), 368, 340; ν_{max} (KBr) 3400 (OH), 1705 cm^{-1} (C=O); [θ] (CH_3OH) −2556° (283 nm); NMR 3.84 (m, 1, CHOH), 0.75 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{26}H_{42}O_2$: C, 80.83; H, 10.88. Found: C, 80.66; H, 10.79

5,10-Seco-19-norcholest-5-yne-3,10-dione (1a). 3β-Hydroxy-5,10-seco-19-norcholest-5-yn-10-one (**6a,** 163 mg, 0.00042 mol) was dissolved in acetone (50 ml) and oxidized with excess Jones reagent for 10 min at ambient temperature. The mixture was diluted with H_2O and extracted with $CHCl_3$. Drying (Na_2SO_4) and removal of the solvent in vacuo gave an oil which was crystallized from MeOH–EtOEt to give **1a** (77 mg, 0.0002 mol): mp 94–96°; mass spectrum *m/e* 384 (M^+), 269, 256, 299; [θ] (dioxane) −6696° (287 nm); ν_{max} (KBr) 1705 cm^{-1}; NMR 0.75 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{26}H_{40}O_2$: C, 81.25; H, 10.42. Found: C, 81.49; H, 10.53

3β,17β-Diacetoxyestr-5(10)-en-6-one (3b). Compound **3b** was prepared in a manner identical with that for **3a** as described above: mp 116–118°; mass spectrum *m/e* 374 (M^+), 332, 314, 286; λ_{max} (MeOH) 245 nm (ε 11078); [θ] (CH_3OH) +2372° (330 nm); ν_{max} (KBr) 1730 (ester C=O), 1660 (C=O), 1620 cm^{-1} (C=C); NMR 5.11 (m, 1, CHOAc), 4.66 (m, 1, CHOAc), 2.02 (s, 3, CH_3CO), 2.00 (s, 3, CH_3CO), 0.86 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.58; H, 8.02. Found: C, 70.31; H, 8.14.

3β,17β-Diacetoxy-5β,10β-oxidoestran-6-one (4b). A mixture of 3β,17β-diacetoxyestr-5(10)-en-6-one (**3b,** 3.74 g, 0.01 mol) and 85% *m*-chloroperbenzoic acid (5.74 g, 0.028 mol) was refluxed in benzene (300 ml) for 1 hr. The cooled solution was washed successively with water, 5% $NaHCO_3$, and water and dried (Na_2SO_4). The residue crystallized from MeOH, after removal of the solvent in vacuo, to give pure epoxide **4b.** Chromatography of the mother liquor on silica gel (elution with 8% EtOAc–$CHCl_3$) gave additional **4b,** total yield 2.7 g (0.0067 mol, 67%): mp 245–247°; mass spec-

trum m/e 390 (M^+), 330, 314, 302, 286; ν_{max} 1725 (ester C=O), 1705 (C=O), 1250 cm^{-1} (ester); [θ] (CH_3OH) −8506° (306 nm); NMR (C_6D_6) 4.60 (m, 2, CHOAc), 2.06 (s, 3, CH_3CO) 2.01 (s, 3, CH_3CO), 0.84 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74. Found: C, 67.65; H, 7.66.

3β,17β-Diacetoxy-5β,10β-oxidoestran-6-one (4b) from 3β,6β,17β-Triacetoxyestr-5(10)-ene (10). To a solution of the triacetate **10** (1.0 g, 0.0026 mol) in acetone (40 ml) at 25° was added Jones reagent (1.2 ml) with swirling. After 10 min, excess reagent was destroyed by dropwise addition of MeOH, and the reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried (Na_2SO_4), and evaporated in vacuo to give a solid residue which was chromatographed on silica gel (elution with $CHCl_3$–EtOAc, 9:1). The first fractions contained starting material (**10**, 119 mg), and later fractions contained the oxido ketone **4b** (200 mg), mp 247° (from MeOH), identical in all respects (ir, NMR, TLC, MS) with an authentic sample of **4b**.

3β,17β-Diacetoxy-5,10-secoestr-5-yn-10-one (5b). A mixture of 3β,17β-diacetoxy-5β,10β-oxidoestran-6-one (**4b**, 2.0 g, 0.005 mol) and *p*-toluenesulfonyl hydrazide (1.24 g, 0.0067 mol) was stirred for 6 hr in 1:1 $CHCl_3$–AcOH (150 ml) at ambient temperature. The $CHCl_3$ extract, after dilution with water, was washed with 5% $NaHCO_3$ and water and dried (Na_2SO_4). Removal of the solvent in vacuo and crystallization of the residue (MeOH) gave **5b** as plates (1.7 g, 0.0045 mol, 90%): mp 204–206°; mass spectrum m/e 374 (M^+), 332, 316, 304, 286; ν_{max} 1724 (C=O), 1250 cm^{-1} (ester); [θ] (CH_3OH) −2710° (284 nm); NMR 4.62 (m, 2, CHOAc), 2.01 (s, 6, CH_3CO at C-3 and C-17), 0.88 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{22}H_{30}O_5$: C, 70.56; H, 8.08. Found: C, 70.70; H, 7.94.

3β,17β-Dihydroxy-5,10-secoestr-5-yn-10-one (6b). The diacetate **5b** (374 mg, 0.001 mol) was stirred at ambient temperature for 3 hr in 3% methanolic KOH (75 ml). Concentration in vacuo after the addition of saturated NaCl (40 ml), extraction with $CHCl_3$, drying (Na_2SO_4), and removal of the solvent gave the pure diol **6b**. Crystallization from hexane–EtOH gave **6b** as needles (280 mg, 0.0096 mol, 96%): mp 205–207°; mass spectrum m/e 290 (M^+), 272, 262, 254, 244; ν_{max} (KBr) 3440 (OH), 1725 cm^{-1} (C=O); [θ] (CH_3OH) −2744° (283 nm); NMR (Me_2SO-d_6) 4.85 (m, 1, CHOH), 4.57 (m, 1, CHOH), 0.78 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.21; H, 8.99.

5,10-Secoestr-5-yne-3,10,17-trione (1b). The 3β,17β-diol **6b** (186 mg, 0.0006 mol) was dissolved in acetone (40 ml) and oxidized with excess Jones reagent for 5 min at ambient temperature. The reaction mixture was diluted with water and extracted with $CHCl_3$. The residue obtained after removal of solvent was crystallized from petroleum ether–$CHCl_3$ to give **1b** as plates (98 mg, 0.00034 mol, 53%): mp 163–166°; mass spectrum m/e 286 (M^+), 271, 258, 243, 230, 215; ν_{max} 1730 cm^{-1} (C=O); [θ] (MeOH) +4607° (305 nm), −3016° (277 nm), +1426° (248 nm); NMR 0.93 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.49; H, 7.74; 0, 16.77. Found: C, 75.25; H, 7.61; 0, 16.67.

3β,10β,17β-Trihydroxy-5α-estran-6-one 3β,17β-Diacetate (9). A mixture of 3β,17β-diacetoxy-5β,10β-oxidoestran-6-one (**4b**, 800 mg, 0.0021 mol) and chromous acetate[15] (3.4 g, ca. 0.2 mol) was stirred at ambient temperature for 24 hr in 90% aqueous acetone (150 ml) under an atmosphere of argon. The reaction mixture was diluted with water and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel (elution with 11% EtOAc in $CHCl_3$). Early fractions gave unchanged oxido ketone **4b** followed by enone **3b**. The most polar fractions gave the desired β-hydroxy ketone **9** (75 mg, 0.00019 mol, 9%): mp 196–199°; mass spectrum m/e 392 (M^+), 376, 349, 314, 304, 272; ν_{max} (KBr) 3480 (OH), 1735 cm^{-1} (C=O); [θ] (CH_3OH) −5078° (288 nm). Calcd for $C_{22}H_{32}O_6$: m/e 392.21900. Found: m/e 392.22510.

3β,20β-Diacetoxy-19-norpregn-5(10)-en-6-one (3c). Compound **3c** was prepared in a manner identical with that for **3a** as described above: mp 126–127° (from acetone–light petroleum); mass spectrum m/e 402 (M^+), 342, 300, 254; λ_{max} (MeOH) 247 nm (ϵ 10435); [θ] (MeOH) +2747° (330 nm); ν_{max} ($CHCl_3$) 1725 (ester C=O), 1660 (C=O), 1625 cm^{-1} (C=C); NMR 4.82 (m, 2, CHOAc), 2.0 (s, 6, CH_3CO), 0.65 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.60; H, 8.26.

3β,20β-Diacetoxy-5β,10β-oxidopregnan-6-one (4c). 3β,20β-Diacetoxy-19-norpregn-5(10)-en-6-one (**3c**, 250 mg, 0.00062 mol) was dissolved in MeOH (18 ml) containing 30% H_2O_2 (1.25 ml) and 5 *N* NaOH (1.25 ml). After stirring for 3 hr at ambient temperature the reaction mixture was diluted with brine and extracted with $CHCl_3$. The extract was washed with 5% $NaHSO_3$, dried with Na_2SO_4, and removed in vacuo. The residue was acetylated pyridine–acetic anhydride) and worked up in the usual manner. Crystallization from hexane–EtOH afforded the pure oxide **4c** (186 mg, 0.00045 mol, 72%): mp 143–145°; mass spectrum m/e 418 (M^+), 360, 358, 342, 340, 330, 298, 270; ν_{max} 1740 (ester C=O), 1700 cm^{-1} (C=O); [θ] (CH_3OH) −9000° (307 nm); NMR 4.68 (m, 2, CHOAc), 2.00 (s, 6, CH_3CO), 1.14 (d, 3, J = 6 Hz, 21-CH_3), 0.68 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{24}H_{34}O_6$: C, 68.87; H, 8.19. Found: C, 68.71; H, 8.11.

3β,20β-Diacetoxy-5,10-seco-19-norpregn-5-yn-10-one (5c). A mixture of 3β,20β-diacetoxy-5β,10β-oxidopregnan-6-one (**4c**, 150 mg, 0.00036 mol) and *p*-toluenesulfonyl hydrazide (78 mg, 0.0043 mol) was stirred for 6 hr at ambient temperature in 1:1 $CHCl_3$–AcOH (15 ml). The reaction mixture was diluted and extracted with $CHCl_3$. The extract was washed successively with H_2O, 5% $NaHCO_3$, and H_2O. The residue obtained after drying (Na_2SO_4) and removal of solvent was crystallized from hexane–acetone to give **5c** as plates (105 mg, 0.00026 mol, 72%): mp 119–120°; mass spectrum m/e 402 (M^+), 360, 358, 342, 340, 330, 298, 270; ν_{max} 1735 cm^{-1} (C=O); [θ] (CH_3OH) −2098° (283 nm); NMR 4.80 (m, 2, CHOAc), 2.05 (s, 3, CH_3CO), 2.00 (s, 3, CH_3CO), 1.15 (d, 3, J = 6 Hz, 21-CH_3), 0.80 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.53; H, 8.64.

3β,20β-Dihydroxy-5,10-seco-19-norpregn-5-yn-10-one (6c). The diacetate **5c** (30 mg, 0.000075 mol) was refluxed in 3% methanolic KOH (7 ml) for 2 hr. The reaction mixture was diluted with brine, extracted with $CHCl_3$, dried (Na_2SO_4), and concentrated in vacuo to give **6c** (23 mg, 0.00072 mol, 96%): mp 180–182° (from hexane–EtOH); mass spectrum m/e 318 (M^+), 300, 285, 282, 272; ν_{max} (KBr) 3500 (OH), 1720 cm^{-1} (C=O); [θ] (MeOH) −3067° (283 nm); NMR 3.74 (m, 2, CHOH), 1.09 (d, 3, J = 6 Hz, 21-CH_3), 0.83 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{20}H_{30}O_3$: C, 75.43; H, 9.50. Found: C, 75.38; H, 9.50.

5,10-Seco-19-norpregn-5-yne-3,10,20-trione (1c). 3β,20β-Dihydroxy-5,10-seco-19-norpregn-5-yn-10-one (**6c**, 140 mg, 0.00044 mol) was dissolved in acetone (50 ml). Excess Jones reagent was added and the mixture was stirred for 5 min at ambient temperature. The residue obtained after dilution with water and extraction with $CHCl_3$ was crystallized from hexane–EtOH to give **1c** (75 mg, 0.00024, 54%): mp 156–159°; mass spectrum m/e 314 (M^+), 286, 271; ν_{max} (KBr) 1705 cm^{-1}; [θ] (dioxane) +4737° (301 nm); NMR 2.14 (s, 3, 21-CH_3CO), 0.75 ppm (s, 3, 18-CH_3).

Anal. Calcd for $C_{20}H_{26}O_3$: C, 76.40; H, 8.34; 0, 15.66. Found: C, 76.22; H, 8.21; 0, 15.97.

Acknowledgments. It is a pleasure to acknowledge helpful discussions with Dr. P. Talalay and Dr. D. Covey. Excellent technical assistance was provided by J. Merritt, G. Zeller, and F. Toffel. Thanks are also due to Dr. H. Ziffer for valuable assistance in obtaining the CD data.

Registry No.—**1a**, 57237-48-6; **1b**, 26012-92-0; **1c**, 55512-68-0; **2a**, 750-59-4; **2b**, 1249-36-1; **2c**, 6764-88-1; **3a**, 50888-45-4; **3b**, 15335-35-0; **3c**, 57214-99-0; **4a**, 57215-00-6; **4b**, 57215-01-7; **4c**, 57215-02-8; **5a**, 57237-49-7; **5b**, 57215-03-9; **5c**, 57215-04-0; **6a**, 57215-05-1; **6b**, 57215-06-2; **6c**, 57215-07-3; **7**, 33487-93-3; **8**, 57215-08-4; **9**, 57215-09-5; **10**, 57215-10-8; chromium trioxide–pyridine complex, 55960-78-6; H_2O_2, 7722-84-1; *m*-chloroperbenzoic acid, 937-14-4; *p*-toluenesulfonyl hydrazide, 1576-35-8; chromous acetate, 628-52-4.

References and Notes

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Krukovine, a New Bisbenzylisoquinoline Alkaloid from *Abuta splendida* Krukoff and Moldenke

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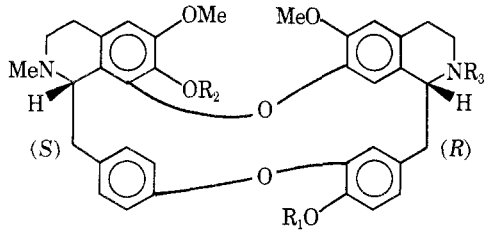
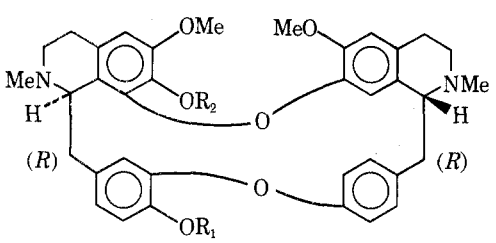
Received July 22, 1975

The bisbenzylisoquinoline alkaloids aromoline, homoaromoline, and krukovine have been found to be the major components of *Abuta splendida* Krukoff and Moldenke. Krukovine has been assigned structure **4** on the basis of both spectroscopic evidence and chemical degradation.

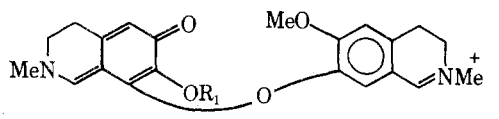
In a continuing search for natural anticancer agents, we have been studying some hitherto unexamined sources of bisbenzylisoquinoline alkaloids, in particular, plants of the genus *Abuta* (*Menispermaceae*).[1] We now wish to report the isolation of the major alkaloidal constituents of the Amazonian species, *Abuta splendida*.[2,3] These include the known bisbenzylisoquinolines aromoline (**1**) and homoaromoline (**2**) as well as the new alkalodd krukovine, which has been shown to have structure **4**.



1, $R_1 = R_2 = H$; $R_3 = Me$
2, $R_1 = Me$; $R_2 = H$; $R_3 = Me$
3, $R_1 = R_2 = R_3 = H$



4, $R_1 = R_2 = H$
5, $R_1 = R_2 = Me$
6, $R_1 = R_2 = CD_3$



7, $R_1 = CD_3$

Aromoline (**1**)[4] was obtained as tiny, colorless prisms, mp 182–183°. Positive identification of this alkaloid was made by direct comparison (ir, mixture melting point, TLC) with an authentic sample obtained by the N-methylation of natural daphnoline (**3**).

Homoaromoline (**2**)[5] was obtained as tiny, white needles, mp 236–237°. It was found to be identical (TLC, mixture melting point) with an authentic sample.

Krukovine (**4**) crystallized from chloroform as colorless prisms of the chloroform solvate, mp 182–183°. The composition $C_{36}H_{38}O_6N_2$ was determined by high-resolution mass spectrometry.

The infrared spectrum of krukovine showed a band at 3400 cm^{-1}, attributable to a nonassociated phenolic group. The NMR spectrum of krukovine showed the presence of two aromatic methoxyls at δ 3.30 and 3.73, as well as two methylimino groups at δ 2.28 and 2.58. Of the ten aromatic protons present five were clearly discernible, one as a singlet at δ 5.97 and four as a pair of doublets at δ 7.11 (d, J = 8 Hz, 2 H) and 7.32 (d, J = 8 Hz, 2 H).

Treatment of krukovine with excess diazomethane, followed by crystallization from acetone, afforded *O,O*-dimethylkrukovine (**5**), mp 125°, confirming the presence of two phenolic functions in the parent molecule. The composition $C_{38}H_{42}O_6N_2$ was determined by high-resolution mass spectroscopy. The corresponding reaction of krukovine with deuteriodiazomethane in dioxane–deuterium oxide[6] yielded the corresponding *O,O*-bistrideuteriomethyl derivative (**6**). A comparison of the NMR spectra of **5** and **6** showed that the new methyl groups of the dimethyl ether **5** are represented by signals at δ 3.18 and 3.93. These values can be assigned to the C-7 and C-12 aromatic methoxyls of a normal head-to-head dimer, since it has been pointed out that a methoxyl at C-7 is highly shielded, while a methoxyl at C-12 (or C-12′) appears in the usual range.[7]

The mass spectrum of krukovine is typical of that of a bisbenzylisoquinoline alkaloid containing both head-to-head and tail-to-tail ether bridges.[8] Thus, a weak peak at M − 107 occurs in the spectra of krukovine (**4**), its dimethyl ether **5**, and the deuterated dimethyl ether **6**. Furthermore, a weak peak at M − 137 is observed in the spectrum of the dimethyl ether **5**, which is shifted to M − 140 in the spectrum of the corresponding deuterated derivative **6**, whereas this feature is absent in the spectrum of krukovine itself. These latter peaks are characteristic of a tail-to-tail diphenyl ether system containing one methoxyl substituent. This requires that one of the phenolic groups of **4** be located on the tail-to-tail diphenyl ether system, and that the second phenolic group of **4** be located on one of the isoquinoline units. In accord with this general formulation, the very strong peak of krukovine representing the linked isoquino-