room temperature for 3 hr under anhydrous conditions. Solvents were removed under reduced pressure, and diethyl ether was added to the residue to precipitate the mixed anhydride XVII. The mixture was kept at 0° for 1 hr and the ethyl ether was removed by decantation. Dioxane (0.2 ml) was added to the precipitated material and the solution concentrated to a syrup under reduced pressure. A solution of di(tri-*n*-butylammonium) pyrophosphate (0.4 mmol) in pyridine (0.15 ml) was added and the mixture was shaken and kept at room temperature for 45 min. Pyridine was removed under reduced pressure and anhydrous ethyl ether was added. The precipitate was dissolved in water and the solution applied to a column (2 × 20 cm) of DEAE-cellulose in the bicarbonate form. Elution was carried out with a linear gradient of water (1 l.) and 0.4 *M* triethylammonium bicarbonate (1 l., pH 7.5) and 10-ml fractions were collected. The starting material (XIV; 23% recovery) was eluted in fractions 63–98. The phosphonylphosphate XVIII (210 OD_{260} units; 7% yield) and the phosphonylpyrophosphate XIX (1560 OD_{260} units; 52% yield) were eluted as symmetrical peaks in the elution diagram in fractions 99–109 (0.18 *M* salt) and 111–158 (0.25 *M* salt), respectively. Fractions 111–158 were evaporated at 20° (12 mm), and the residue was dried by addition and evaporation of 15 ml of ethanol. This was followed by two evaporations of 0.1 ml of triethylamine in 5 ml of 50% ethanol, and one of 10 ml of ethanol. A solution of the residue in 3 ml of dry methanol was mixed with 2 ml of 1 *M* NaI in dry acetone, and the sodium salt was precipitated by addition of 10 ml of acetone. The salt was washed with acetone (four 20-ml portions) and dried at 20° (2 mm). The residue was freed from residual XVIII by ascending chromatography on acid-washed Whatman 3 MM paper with repeated development in isobutyric acid–1 *M* NH_4OH (100:60). The compound (XIX) obtained by lyophilization of the aqueous eluate had $\lambda_{max}^{H_2O}$ 258 nm and was homogeneous on paper chromatography, electrophoresis, and PEI-cellulose chromatography with properties similar to those of ATP (Table I).

***N*,*N*-Dibenzoyl-5′-deoxy-5′-(diethoxyphosphinyl)carboethoxymethyl-2′,3′-*O*-isopropylideneadenosine (XX).** The reaction was carried out as described for the preparation of VII, utilizing 36 mg (1.5 mmol) of sodium hydride, 403 mg (1.8 mmol) of triethyl phosphonoacetate (IIa), and 625 mg (1 mmol) of VI in 15 ml of DMSO, at 65–75° for 2 hr. Tlc of the chloroform extract with five successive developments in system D showed the presence of six or more ultraviolet-absorbing components which were quantitated spectrophotometrically and had respectively R_f 0.92 (VI) (28% yield), 0.73 (*N*-monobenzoyl-2′,3′-*O*-isopropylidene-5′-iodo-5′-deoxyadenosine, IX) (19% yield), 0.51 (the dibenzoyl phosphonate XX) (6% yield), 0.46 (monobenzoyl-2′,3′-*O*-isopropylidene-3,5′-cycloadenosine, X) (25% yield), 0.29 (monobenzoyl analog XXI of XX) (2% yield), and 0.01 (unidentified product).

A sample was purified by preparative tlc on silica gel as described above. Material of R_f 0.51 was eluted with chloroform and the extract was filtered through Celite and evaporated to a solid which afforded a white amorphous powder (37 mg, 5.1%) from chloroform solution after addition of petroleum ether. This product showed λ_{max}^{EtOH} 247 nm (ε 39,000) and 272 (20,200) and mp 92–99° dec. It exhibited ir absorption at 1720 (C=O) and at 1250 cm^{-1} (P=O).

Anal. Calcd for $C_{35}H_{40}N_5O_{10}P$: C, 58.25; H, 5.54; N, 9.70. Found: C, 57.85; H, 5.93; N, 10.21.

The component of the reaction mixture which had R_f 0.29 was concluded to be XXI because of its relatively low R_f value compared to that of XX and because it had a negative Beilstein halogen test and an absorption maximum in EtOH of 280 nm indicative of a mono-*N*-benzoyladenosine.

Enzyme Kinetic Studies. All assays were carried out by measuring the rate of change of optical density (OD) at a suitable wavelength in a Cary Model 15 spectrophotometer using 1-cm cells containing a final volume of 1 ml at 20°. In all systems the initial velocity with AMP or XIV as substrate was linear and proportional to the concentration of primary enzyme and independent of the concentration of secondary enzymes used in coupled assays. Michaelis, inhibition constants, and maximal velocity (V_{max}) values were determined graphically by the method of Lineweaver and Burk.[39]

Adenylate deaminase (Sigma, grade IV, from rabbit muscle) activity was measured by following the decrease in absorbance at 265 nm in a system containing 1 ml of 0.01 *M* citrate buffer (pH 6.5) and 25 m*M* KCl on addition of 0.03 and 0.93 μg (for AMP and XIV, respectively) of AMP deaminase which was diluted into 1 *M* KCl prior to use. The decrease in OD was measured at 265 nm where Δε for the conversion was 6600.

5′-Nucleotidase activity (Sigma, grade II, *Crotalus adamanteus* venom) was measured by following the decrease in absorbance at 265 nm in a coupled assay with adenosine deaminase (20 μg), 5′-nucleotidase (1.8 μg), and AMP and XIV in 1 ml of 0.1 *M* Tris hydrochloride (pH 8.5). Measurement and calculation of Δε were identical with AMP deaminase.

Each adenylate kinase (Boehringer, pig, and rabbit muscles) was studied in 1 ml of 0.1 *M* Tris hydrochloride (pH 7.6) containing $MgSO_4$ (1 m*M*), KCl (0.1 *M*), PEP cyclohexylammonium salt (0.87 m*M*), ATP (0.28 m*M*), NADH sodium salt (0.38 m*M*), adenylate kinase (0.43 μg for AMP and 40 μg for XIV), pyruvate kinase (Boehringer, 4 μg for AMP and 400 μg for XIV), and lactic dehydrogenase (Sigma, 4 μg for AMP and 400 μg for XIV). After an initial period of 30 min during which the OD change at 340 nm reached a constant value, the reaction was started by the addition of either AMP or XIV.

Acknowledgments. The authors are indebted to Dr. J. G. Moffatt for a gift of 6′-deoxyhomoadenosine-6′-phosphonate, to Dr. R. K. Preston for the synthesis of starting materials, and to Dr. F. Kappler for obtaining pmr spectra and contributing to their interpretation.

(39) H. Lineweaver and D. J. Burk, *J. Amer. Chem. Soc.*, **56**, 658 (1934).

Communications to the Editor

Direct Formation of the Steroid Nucleus by a Nonenzymic Biogenetic-Like Cyclization.[1] Preparation of the Cyclization Substrate

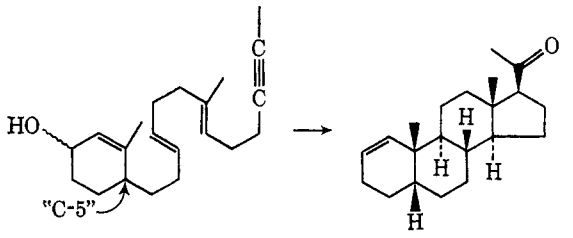
Sir:

The aim of the present work was to synthesize the trienynol **1** and to study its cyclization. This substrate incorporates a cyclohexenol moiety of a type known[2] to initiate facile stereoselective cyclization so as to produce an A/B cis ring fusion. At the same time the substrate contains the terminal methylacetylenic residue which has been shown[3] to yield the C/D trans 6/5 ring system, so that in the event of complete cyclization the product would be the tetracyclic substance **2**. Finally, the substrate **1** has the advantage over previous systems that have been examined in that it has an asymmetric center at C-5 (steroid numbering) rendering it susceptible to obtention in its enantiomeric forms which

(1) For the previous paper in this series see W. S. Johnson, M. B. Gravestock, R. J. Parry, and D. A. Okorie, *J. Amer. Chem. Soc.*, **94**, 8604 (1972).

(2) W. S. Johnson, P. J. Neustaedter, and K. K. Schmiegel, *ibid.*, **87**, 5148 (1965).

(3) (a) W. S. Johnson, M. B. Gravestock, R. J. Parry, R. F. Myers, T. A. Bryson, and D. H. Miles, *ibid.*, **93**, 4330 (1971); (b) W. S. Johnson, M. B. Gravestock, and B. E. McCarry, *ibid.*, **93**, 4332 (1971).



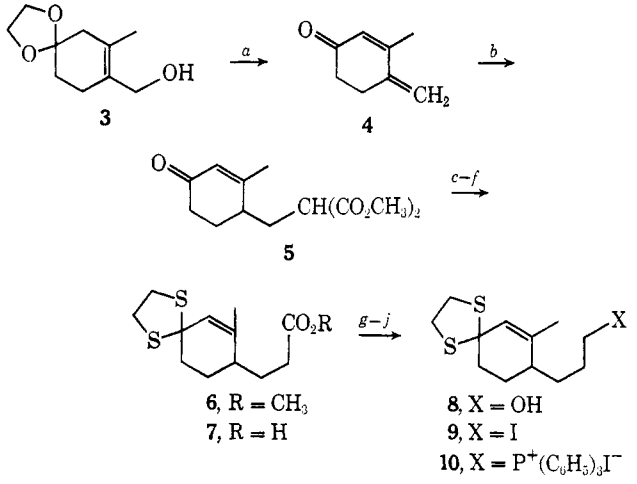
1 2

would be expected to yield optically active cyclization products. All of these objectives have now been realized and we are herewith disclosing the first example in which the complete steroid nucleus is produced as the primary product of a nonenzymic olefinic cyclization, and the first case of the stereospecific nonenzymic formation of an optically active tetracyclic substance which is easily convertible into a natural steroid. The present paper describes the syntheses of the *dl*, *d*, and *l* forms of the cyclization substrate **1** and the subsequent paper[4] gives an account of the cyclization studies of these stereoisomers.

The synthesis of the trienynol **1** that was envisaged (see Scheme II) involved, as the key (convergent) step, the reaction of the phosphorane **11** with the known[3b] aldehyde **12**. This approach afforded the advantageous possibility of effecting optical resolution at an early stage of the synthesis, namely with the thioketal acid **7**.

The synthesis of the phosphorane **11** was accomplished as depicted in Scheme I. The known ketal

Scheme I



[a] 10% HCl, THF, 4 hr, 20°. [b] 3.3 mol equiv of $CH_2(CO_2CH_3)_2$, 0.08 mol equiv of $NaOCH_3$, CH_3OH, 19 hr, 20°. [c] HOAc–H_2O–concentrated HCl (5:5:1), 19-hr reflux. [d] CH_2Cl_2, CH_3OH, *p*-$CH_3C_6H_4SO_3H$, 17-hr reflux. [e] To give **6**: $CHCl_3$, $HSCH_2CH_2SH$, $BF_3 \cdot Et_2O$, 5.5 hr, 20°. [f] To give **7**: CH_3OH, H_2O, KOH, 24 hr, 20°. [g] To give **8**: 1.3 mol equiv of $NaAlH_2(OCH_2CH_2OCH_3)_2$, THF, 4 hr, 0°. [h] 1.4 mol equiv of *p*-$CH_3C_6H_4SO_2Cl$, C_5H_5N, 2 hr, 0°. [i] To give **9**: NaI, acetone, $(i\text{-}Pr)_2NEt$, 2.5 hr, 22°. [j] To give **10**: 1.4 mol equiv of $P(C_6H_5)_3$, CH_3CN, $(i\text{-}Pr)_2NEt$, 10 hr, 50°.

alcohol **3**,[5] on treatment with hydrochloric acid, underwent hydrolysis and dehydration to give the dienone **4**[6] in 87% overall yield from Hagemann's ester. A sample was distilled at 96–98° (15 mm) (*Anal.* Found: C, 78.7; H, 8.4). The Michael condensation of malonic ester with the undistilled dienone **4** proceeded by 1,6 addition[7] to yield the product **5** which, without purification of any intermediates, was successively treated so as to effect hydrolysis, decarboxylation, esterification,[8] and conversion to the thioketal **6**. This last substance on short-path distillation at 180° (0.025 mm) was obtained in 53% overall yield from the dienone **4**. That the olefinic bond of this ketal ester (*Anal.* Found: C, 57.2; H, 7.3; S, 23.6) was in the position shown in formula **6** was evident from the nmr spectrum[6] which showed absorption at δ 1.68 ppm (3 H, s) for the vinyl methyl group and at 5.60 (1 H, s) for the vinyl proton.

Saponification of the ester **6** afforded the free acid **7** (100% yield). Short-path distillation at 173° (0.015 mm) gave a colorless oil (*Anal.* Found: C, 55.9; H, 7.0; S, 24.9). Resolution was effected by three recrystallizations from ethyl acetate of the salt derived from the crude acid **7** and *d*-α-methylbenzylamine. Regeneration of the free acid gave *d*-**7**, $[\alpha]D$ +13.7°.[9] After hydrolysis of the *l*-enriched mother liquor salt to the free acid, the *l* enantiomer was obtained by conversion to and recrystallization of the *l*-α-methylbenzylamine salt. Hydrolysis afforded *l*-**7**, $[\alpha]D$ −14.2°.[9] (It is noteworthy that the unnatural *l* isomer is easily racemized, under thioketalization conditions, and thus can be used for production of the *d* isomer.) Hydride reduction of the resolved acids, *d*-**7** and *l*-**7**, as well as the racemic ester *dl*-**6** gave the corresponding alcohols *d*-**8**, *l*-**8**, and *dl*-**8**[6] in quantitative yield. A sample of *dl*-**8** was purified by tlc followed by short-path distillation at 180° (0.05 mm) (*Anal.* Found: C, 59.2; H, 8.2; S, 26.2). These forms of alcohol **8** were transformed, *via* the crude *p*-toluenesulfonate and then the iodide **9** (chromatographed on Florisil), into the phosphonium salt **10** which was purified by dissolution in methylene chloride and addition to hexane to precipitate the salt. The yields were 78–85% overall from **8**: *d*-**10**, mp 88–90°, $[\alpha]D$ +5.08°[9] (*Anal.* Found: C, 58.3; H, 5.7; I, 20.7);[6] *l*-**10**, mp 91–93.5°, $[\alpha]D$ −4.79°;[9] *dl*-**10**, mp 87–91.5°. In the formation of the phosphonium salt, it was necessary to keep the reaction temperature from exceeding 50° in order to suppress racemization. The addition of diisopropylethylamine to the reaction mixture kept the olefinic bond in the ring from migrating to the β,γ position.

The conversion of the various forms of the phosphonium salt **10** into the ylide **11** and its stereoselective condensation with the aldehyde **12** were carried out just as previously described for a similar case.[3b] Thus, the *all-trans*-trienyne thioketal **13**[6] was produced in 65–72% yield, after chromatography on Florisil. Analysis by vpc indicated that this product contained <2% of the β,γ-unsaturated thioketal and <1% of the cis,trans isomer. A sample of *d*-**13** was purified by tlc and short-path distillation at 200° (0.010 mm) (*Anal.* Found: C, 73.9; H, 9.0). Hydrolysis of *dl*-**13** by procedure *a*[10] of Scheme II afforded, after short-path

(4) B. E. McCarry, R. L. Markezich, and W. S. Johnson, *ibid.*, **95**, 4416 (1973).

(5) Readily prepared in two steps (ketalization followed by hydride reduction) from commercially available Hagemann's ester: K. E. Harding and K. A. Parker, *Tetrahedron Lett.*, 1633 (1971).

(6) The nmr spectrum at 60 MHz ($CDCl_3$ solvent and TMS internal standard) as well as the ir spectrum were entirely consistent with the assigned structure.

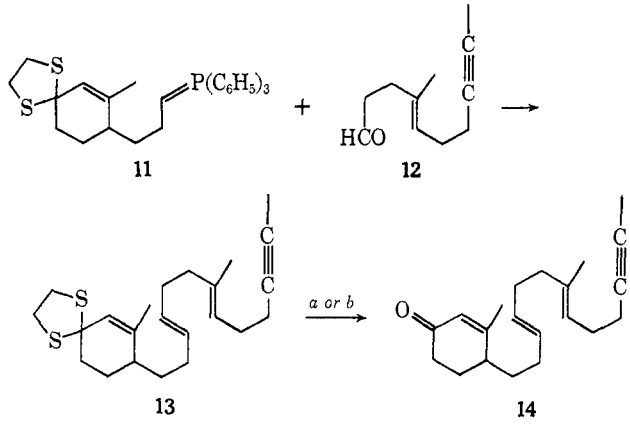
(7) *Cf.* the homologous case: M. Mongrain, J. Lafontaine, A. Bélanger, and P. Deslongchamps, *Can. J. Chem.*, **48**, 3273 (1970).

(8) R. O. Clinton and S. C. Laskowski, *J. Amer. Chem. Soc.*, **70**, 3135 (1948).

(9) Rotations were recorded at 22° using dilute (0.003–0.08 *M*) solutions in chloroform in a 1-dm tube.

(10) *Cf.* M. Fetizon and M. Jurion, *J. Chem. Soc., Chem. Commun.*, 382 (1972).

Scheme II



[a] CH_3CN–H_2O–CH_3I (10:2:1), 11 hr, 50°. [b] For optically active **13**: DMF–H_2O–CH_3I (10:2:1), $CaCO_3$, 44 hr, 22°.

distillation at 160° (0.025 mm), the ketone *dl*-**14**[6] in 93% yield (*Anal.* Found: C, 84.6; H, 10.3). For suppressing racemization during the ketal hydrolysis, buffered conditions (procedure *b*, Scheme II) were required and the yields were lower (40–50%): *d*-**14**, $[\alpha]D$ +58.4°;[9] *l*-**14**, $[\alpha]D$ −58.0°.[9] The *dl*, *d*, and *l* forms of the allylic alcohol **1**[6] were obtained as a mixture of C-2 epimers in quantitative yield by reduction of the various forms of **14** with excess sodium bis(2-methoxyethoxy)aluminum hydride in tetrahydrofuran (2 hr, 0°). A sample of *dl*-**14** was chromatographed on basic alumina (*Anal.* Found: C, 84.0; H, 10.5). The cyclization of the various forms of **1** and proof of structure and configuration of the products are disclosed in the accompanying communication.[4]

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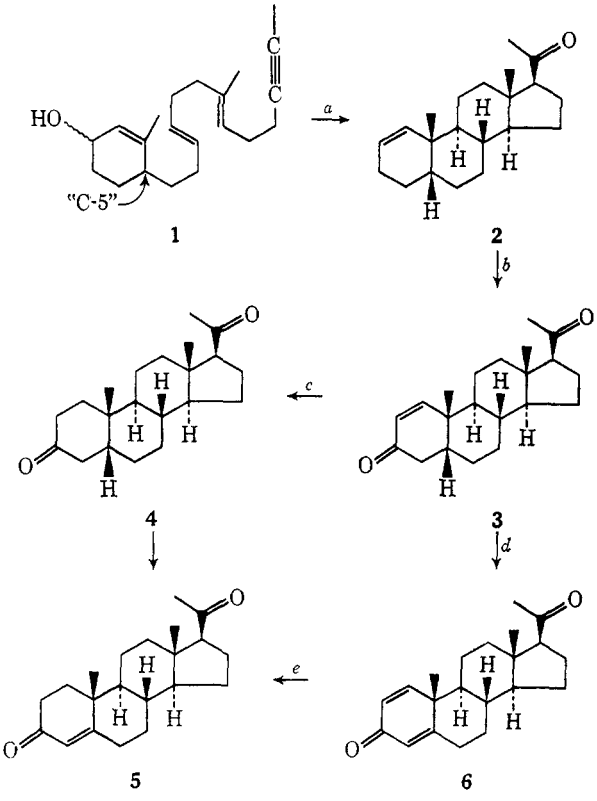
Direct Formation of the Steroid Nucleus by a Nonenzymic Biogenetic-Like Cyclization. Cyclization and Proof of Structure and Configuration of Products

Sir:

In a companion paper,[1] we have disclosed the synthesis of the trienynol **1** in its *dl*, *d*, and *l* forms (as a mixture of C-2 epimers, but essentially optically pure with respect to C-5). The present paper describes the cyclization of these stereoisomers, and the proof of structure and configuration of the products. In the course of this study a totally synthetic pathway to optically active progesterone (**5**) has been realized.

The various forms of the crude alcohol **1** were cyclized as described for a related case[2] except that 1,1-difluoroethane was employed as the solvent (see Scheme I). The reaction mixture was maintained at

Scheme I



[a] CF_2HCH_3, 12% ethylene carbonate, 8% CF_3CO_2H, 1.5 hr, −25°. [b] 7.0 mol equiv of *tert*-butyl chromate, $CCl_2{=}CCl_2$, HOAc, Ac_2O, 50 min, 100°. [c] H_2, 10% Pd/C. [d] 1.5 mol equiv of DDQ, 2 mol equiv of $C_6H_5CO_2H$, toluene, 4 hr, 120°. [e] Rh-$(PPh_3)_3I$, toluene–ethanol (1:1), H_2.

reflux (−25°) for 1.5 hr before being quenched with alkali. Chromatography on Florisil gave a 65% yield of Δ^1-5β-pregnen-20-one (**2**)[3] as an 85:15 mixture of the 17β:17α epimers (shown by vpc). Crystallization from methanol–ethyl acetate afforded the 17β epimer: *dl*-**2**, mp 102.5–103.5°, mass spectrum *m/e* 300 (M^+); *d*-**2**, mp 89.5–90.5°, $[\alpha]D$ +178°[4] (optical purity 100%; see below); *l*-**2**, mp 89.5–90.5°, $[\alpha]D$ −177°.[4] The *dl* form of substance **2** was hydrogenated (after Raney nickel treatment) over 10% palladium-on-carbon to give *dl*-5β-pregnan-20-one, mp 112–113.5°, after crystallization from ethanol (*Anal.* Found: C, 83.5; H, 11.1). The *d* form of **2**, on hydrogenation as above, gave the known[5] *d*-5β-pregnan-20-one. Three recrystallizations from methanol gave colorless needles, mp 114.5–115.5° ($[\alpha]D$ +111°[4]), undepressed on admixture with authentic, naturally derived material, mp 113–115.5°.[6] The ir spectra (KBr) of the two samples were identical.

The optical purities of the cyclization products were determined as follows. A sample of *d*-**2**, $[\alpha]D$ +169°,[4] was hydrogenated as above and the product was chromatographed on Florisil. The rotation of the total dihydro-**2** fraction was $[\alpha]D$ +105°,[4] corresponding to an optical purity of 95.5%. Thus the rotation of

(1) R. L. Markezich, W. E. Willy, B. E. McCarry, and W. S. Johnson, *J. Amer. Chem. Soc.*, **95**, 4414 (1973).
(2) W. S. Johnson, M. B. Gravestock, and B. E. McCarry, *ibid.*, **93**, 4332 (1971).
(3) The nmr spectrum at 60 MHz ($CDCl_3$ solvent and TMS internal standard) as well as the ir spectrum were entirely consistent with the assigned structure.
(4) Rotations were recorded at 22° using dilute (0.003–0.08 *M*) solutions in chloroform in a 1-dm tube.
(5) Reported for naturally derived 5β-pregnan-20-one, mp 114–115°, $[\alpha]^{16-22}D$ +110° ($CHCl_3$): L. Gyermek, J. Iriarte, and P. Crabbé, *J. Med. Chem.*, **11**, 117 (1968).
(6) We wish to thank Dr. J. A. Edwards of Syntex for providing us with this specimen.