cyclopropane in 1.5 ml of tetrahydrofuran was subjected to microhydrogenation over a 15-mg sample of prereduced rhodium on alumina. After 3.5 hr, 6.10 ml of hydrogen had been absorbed. Glpc analysis[25b] at 50° showed the presence of five products with retention times of 3.5, 3.8, 4.0, 4.2, and 4.6 min, with relative areas of 6:1:3:7:2, respectively. The four products with retention times of 3.5, 3.8, 4.0, and 4.2 min, were identified as 3-methylpentane (**20**), 2-ethyl-1-butene (**21**), *trans*-3-methyl-2-pentene (**23**), and *cis*-3-methyl-2-pentene (**22**), respectively, by enrichment with authentic synthetic samples. Glpc analysis on two different capillary columns[25b,c] confirmed that the product with a retention time of 4.6 min was identical with *cis*-1,2,3-trimethylcyclopropane (**19**) obtained from the reduction of 1,2,3-trimethylcyclopropene (**18**).

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Buxus Alkaloids. XIII.[1] A Synthetic Approach to the 9(10→19)*abeo*-Pregnane System[2,3]

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Abstract: When 9β,19-cyclo-5α-pregnane-3,11,20-trione 3,20-diethylene ketal (V) was subjected to the Huang-Minlon modification of the Wolff-Kishner reduction, the expected 11-deoxo compound was not obtained. Instead, two crystalline C-10 epimers, 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-pregnene-3,20-dione 3,20-diethylene ketal (IX) and 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-pregnene-3,20-dione 3,20-diethylene ketal (X), were isolated and characterized. Selective ketal hydrolysis of the diketals with boron trifluoride etherate yielded the respective C-20 ketones, XI and XII. Catalytic hydrogenation of the diketals yielded the dihydro derivatives, XIII and XIV, and hydrolysis afforded the respective dihydro monoketones, XV and XVI, and dihydro diketones, XVII and XVIII. The configurations of IX and X were determined by optical rotatory dispersion measurements of XV–XVIII. The potential significance of the ring B enlargement reaction for the chemical interrelation of the two principal structural types of the *Buxus* alkaloids and for the biogenesis of alkaloids of the 9(10→19)*abeo*-pregnane series are discussed.

In 1962, we reported the elucidation of the structure[4] and configuration[5] of cyclobuxine-D (I), an alkaloid isolated from *Buxus sempervirens* L.[6] Cyclobuxine-D was shown to be the prototype of a new class of steroidal alkaloids which contains the 9β,19-cyclo-5α-pregnane system (II) and has a substitution pattern at C-4 and C-14 which is intermediate in the biogenetic scheme between lanosterol and cholesterol-type steroids. Subsequent studies have characterized many structurally related alkaloids.[7] In 1964, the isolation and characterization of buxenine G[8a] ("norbuxamine"[9]) was reported and this alkaloid was later proven to possess the novel structure and configuration III.[8b] Several additional alkaloids which possess the unusual 9(10→19)*abeo*-5α-pregnane[10] system (IV) have been found.[1,9,11,12]

In the course of studies on the synthesis of analogs of *Buxus* alkaloids which contain the 9β,19-cyclo-5α-pregnane system (II), an unusual Kishner reduction which involves cleavage of a carbocyclic ring has been observed. The reaction constitutes a synthetic approach to derivatives of the 9(10→19)*abeo*-5α-pregnane system (IV).

Examination of a Dreiding model of 9β,19-cyclo-5α-pregnane-3,11,20-trione 3,20-diethylene ketal (V)[13] indicated that the 11-carbonyl group in this molecule is not as hindered as that of a normal steroid nucleus, owing to the absence of a 1,3-interaction with the C-19 methyl group. When the compound was subjected to the Huang-Minlon modification of the Wolff-Kishner reduction, however, the expected 11-deoxo compound was not obtained. The infrared spectrum

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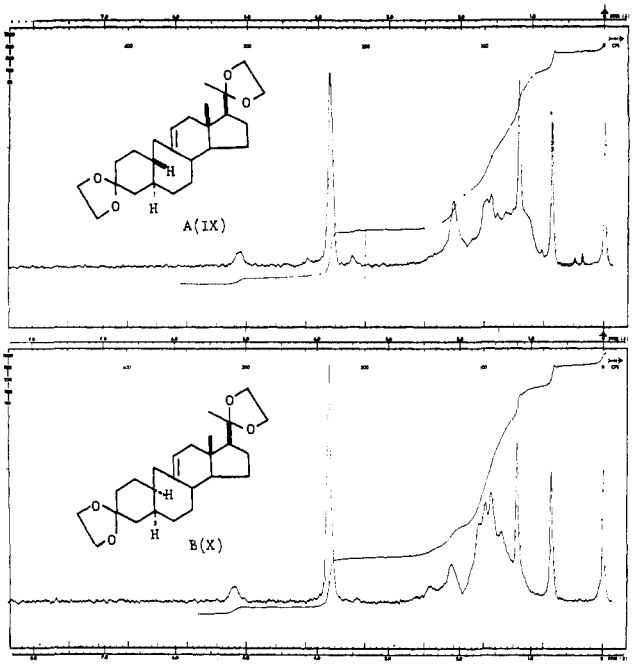
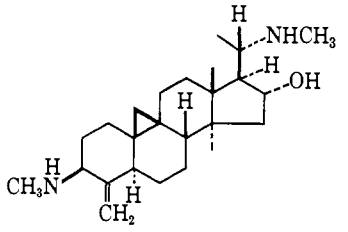


Figure 1. Nmr spectra of compounds A (IX) and B (X) (in CCl_4).

of the crude reaction mixture indicated that complete reduction of the carbonyl group had occurred. Column chromatography on neutral alumina yielded a crystalline product. However, analytical gas chromatography showed that the crystalline product consisted of two components. Careful column chromatography or preparative thick layer chromatography led to separation of the components of the mixture.



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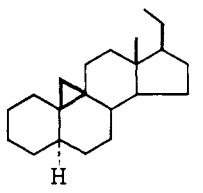
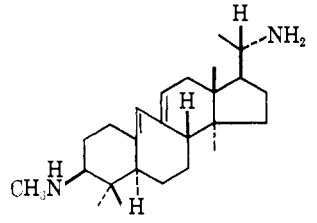
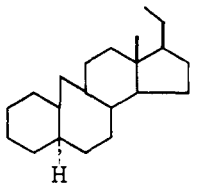


II



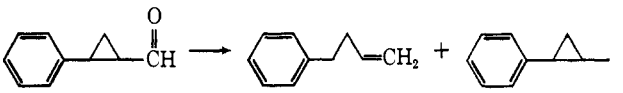
III



IV

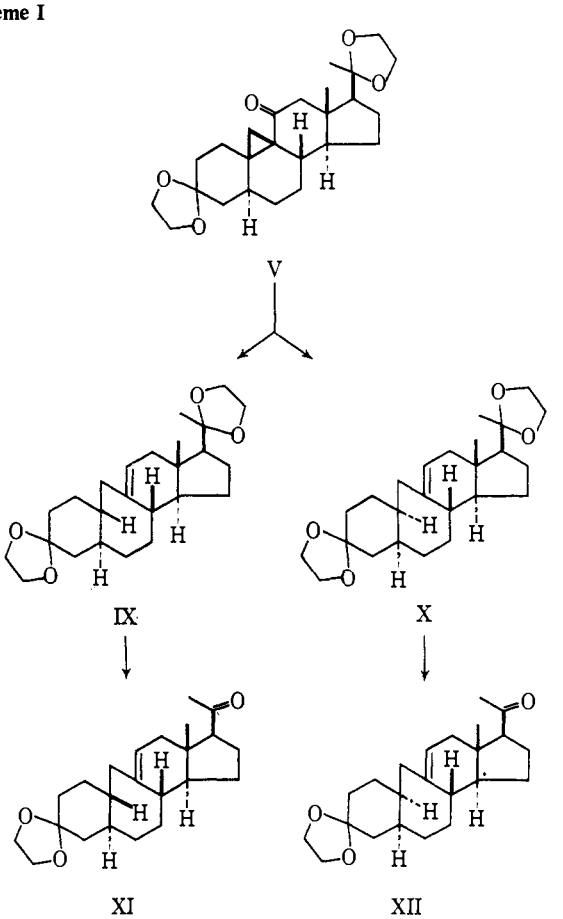
Two crystalline products were obtained, A, $C_{25}H_{38}O_4$, mp 109–110°, 20% yield, and B, $C_{25}H_{38}O_4$, mp 124–126°, 10% yield. The nmr spectra of A and B (Figure 1) indicated that each had two tertiary C-methyl groups, eight ethylene ketal protons, one vinyl proton, and no cyclopropane methylene protons. The latter would have been expected to show clear sharp signals in the absence of the deshielding effect of a C-11 carbonyl group.[14,15] Compounds A and B each decolorized bromine solution, gave a positive test with tetranitromethane, and consumed 1 mole of hydrogen (accompanied by the disappearance of the vinyl proton signal in the nmr spectrum).

Bumgardner and Freeman[16] have recently reported a carbocyclic ring cleavage of 2-phenylcyclopropanecarboxaldehyde (VI) to give 4-phenylbutene-1 (VII) as one of two products and the latter appears to have been the first reported case of carbocyclic ring cleavage during Wolff–Kishner reduction. This type of α-elimination reaction was first observed by Kishner in his work on 2,6-dimethyl-2-hydroxy-3-octanone, when he obtained 2,6-dimethyloctene-2 instead of the expected 2,6-dimethyloctanol-2.[17] Similar α-elimination reactions of α-amino ketones, α-substituted pinacolones, and α-epoxy and α-halo ketones have also been reported.[18–21] Analogy with the precedents and the chemical, chromatographic, and spectral characteristics of A and B led to the conclusion that ring B enlargement had taken place during Wolff–



VI VII VIII

Scheme I



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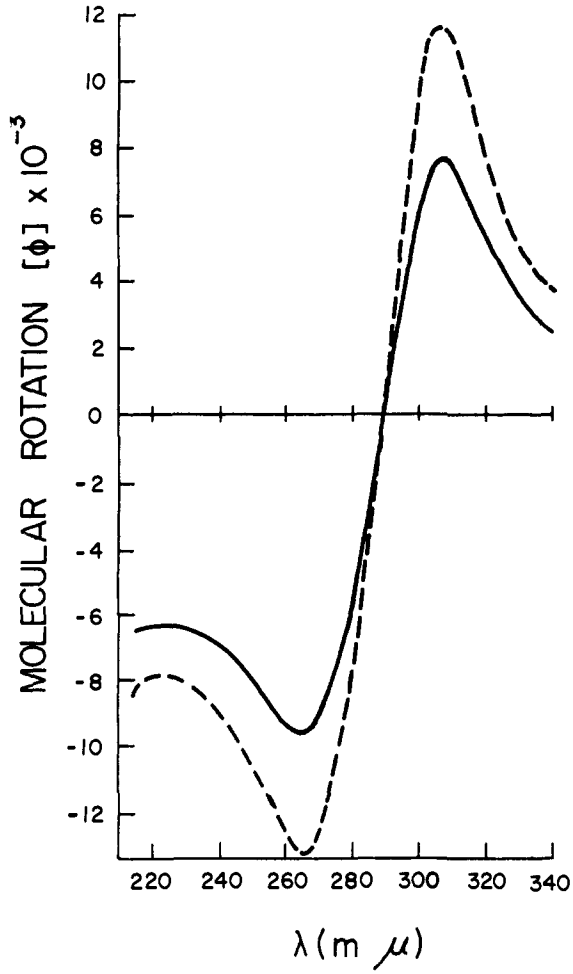


Figure 2. ORD curves for XV (———) and XVII (– – – –).

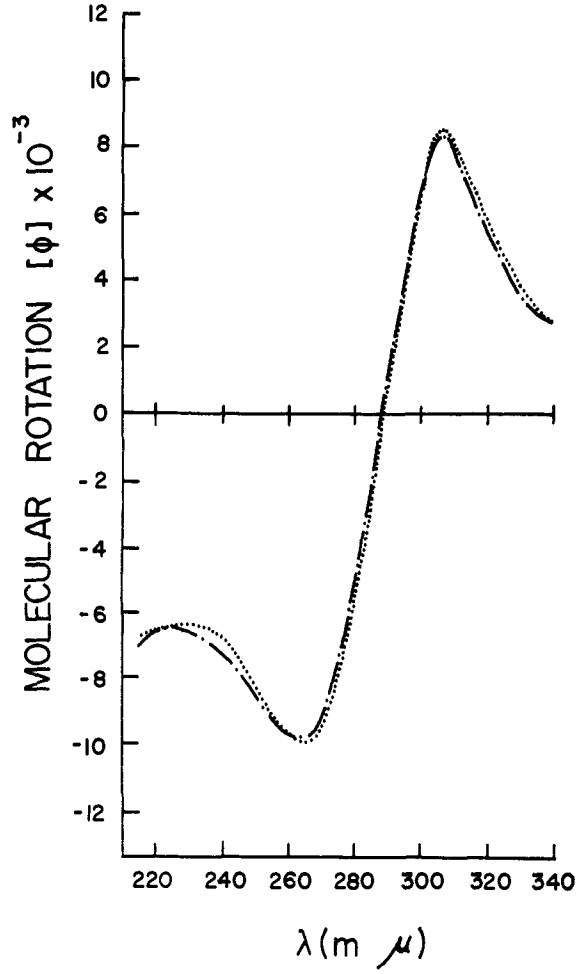
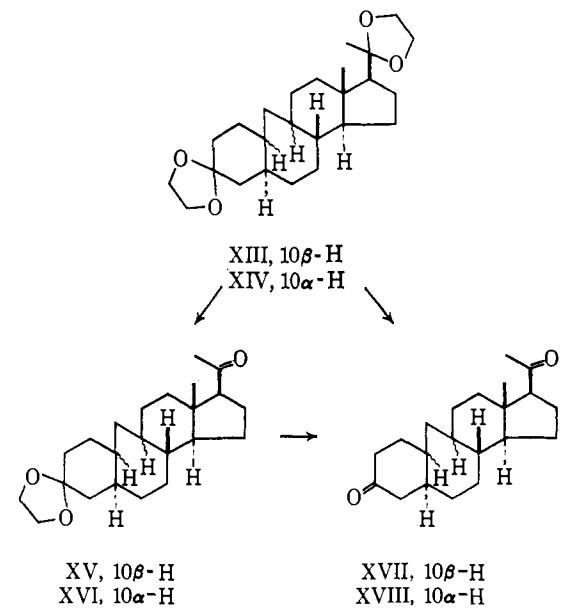


Figure 3. ORD curves for XVI (– · – ·) and XVIII (· · ·).

Kishner reduction of V, affording C-10 epimers which could be represented as IX and X (Scheme I). Selective ketal hydrolysis of IX and X with boron trifluoride etherate yielded the C-20 monoketones, XI, $C_{23}H_{34}O_3$, mp 134–136°, and XII, $C_{23}H_{34}O_3$, mp 90–92°. Attempted further hydrolysis with *p*-toluenesulfonic acid in acetone gave very small yields of incompletely characterized diketones. The spectral properties of the latter diketones supported the view that additional reactions had accompanied the hydrolyses.

The configurations at C-10 in IX and X were determined by optical rotatory dispersion studies of appropriate dihydro derivatives. Catalytic hydrogenation of IX gave XIII, $C_{25}H_{40}O_4$, mp 121–122°. The nmr spectrum showed no vinyl proton signals. Partial deketalization of XIII, by treatment with alumina, gave XV, $C_{23}H_{36}O_3$, mp 136–137° (Scheme II). The infrared spectrum indicated the presence of a carbonyl group and the nmr spectrum showed the presence of one tertiary C-methyl group, the expected downfield shift of the C-21 methyl group, and four ketal protons. Treatment of XV or XIII with *p*-toluenesulfonic acid yielded diketone XVII, $C_{21}H_{32}O_2$, mp 100–101°. Hydrogenation of X gave an amorphous dihydro product (XIV). Selective deketalization of XIV with alumina gave the C-20 monoketone XVI, $C_{23}H_{36}O_3$, mp 118–120°, and the infrared and nmr spectra indicated that XVI contained the same functional groups as XV. Hydrolysis of XIV or XVI with *p*-toluenesulfonic acid in refluxing aqueous acetone gave diketone XVIII, $C_{21}H_{32}O_2$, mp 149–150°.

Scheme II



The optical rotatory dispersion curves of XV and XVI were positive (Figures 2 and 3) as expected, since the 20-carbonyl function in the pregnane system is

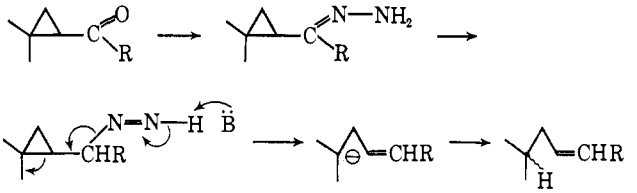
known to show a strong positive Cotton effect.[22,23] When the optical rotatory dispersion curve was determined for the diketone XVII, a marked increase in amplitude was observed. On the other hand, the diketone XVIII showed no appreciable change in amplitude (Figure 3).

In saturated diketones with absorption bands of each chromophore situated more or less at the same wavelength, the Cotton effect is frequently the mere arithmetic sum of both chromophores. This "additivity rule" is applicable only when an absence of vicinal interactions between the two chromophores is assured.[22,24] It has been shown that this rule can be applied for the 6,20-diketo *i*-steroid XIX[25-27] and the tetracyclic triterpene XX.[28] Hence, it is assumed that no vicinal interaction exists between the C-3 and C-20 carbonyl groups in XVII and XVIII.

It has been reported that the ORD curves of 19-nor-5α,10α-androstan-17α-ethyl-17-ol-3-one (XXI)[29] and 19-nor-5α-androstan-17β-ol-3-one (XXII)[30] exhibit plain and strong single positive Cotton effects, respectively. By analogy, A was assigned structure IX, 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-pregnene-3,20-dione diethylene ketal, and isomer B was assigned structure X, 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-pregnene-3,20-dione diethylene ketal.[31]

It has been suggested that the Wolff–Kishner reaction may proceed *via* a hydrazone anion[32-34] which loses nitrogen and takes up a proton to lead to the reduced product. A similar noncyclic stepwise mechanism has been postulated to account for the reduction elimination reaction of α-substituted ketones.[35-38] Leonard and Gelfand proposed that this reaction is a member of the general class of base-catalyzed elimination reactions. It was suggested that the reaction proceeded *via* a diimine and decomposition of the intermediate diimine was pictured as analogous to other planar four-centered eliminations, differing only in that the proton is removed from a group attached to the β-carbon itself.[19] A mechanism which involves intramolecular abstraction of a proton through a cyclic transition state has been advanced to account for the shift of the double bond encountered during Wolff–Kishner reduction of α,β-unsaturated carbonyl containing compounds.[39] It has also been used to explain the remarkable ease with which the hydrazones of α-thienyl carbonyl, *o*-nitrophenyl carbonyl, and 1,2-diketones decomposed to the corresponding Wolff–Kishner reaction products.[34]

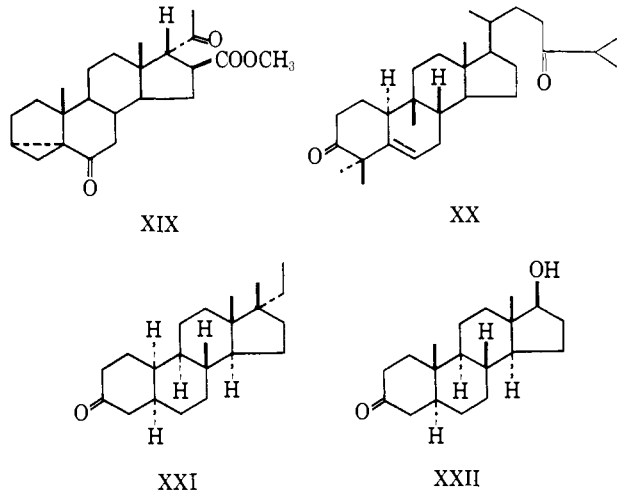
The Kishner reduction of V may proceed *via* a stepwise mechanism to a C-10 carbanion, followed by protonation from either side of the molecule to the observed products. For the present, however, alternative mechanisms, perhaps involving free-radica intermediates, cannot be precluded.



Several new *Buxus* alkaloids which possess the same conjugated cyclopropyl ketone system as in V have recently been isolated.[1,40] By analogy with the work reported herein, Kishner reduction of the latter alkaloids may lead to chemical interrelation with alkaloids of the buxenine-G type.[41] Furthermore, the facile Kishner reduction with ring B enlargement may have significance as an appropriate model for the biosynthesis of alkaloids of the 9(10→19)*abeo*-pregnane series.

Experimental Section[42]

9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-Pregnene-3,20-dione Diethylene Ketal (IX) and 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-Pregnene-3,20-dione Diethylene Ketal (X). A solution of potassium hydroxide (4.8 g)



XIX XX XXI XXII

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(41) R. Goutarel and collaborators (*ibid.*, 789 (1967)) have recently described an elegant alternative chemical interrelation, which proceeds *via* sulfuric acid treatment of an 11-hydroxy-9β,19-cyclo-5α-pregnane alkaloid derivative.
(42) All melting points were determined on a Fisher-Johns melting point apparatus and are corrected. Infrared spectra were determined as KBr pellets (unless otherwise noted), on a Beckman double-beam recording spectrophotometer, Model IR-9 or IR-5A (with polystyrene reference peak at 6.24 μ). All rotations were determined in chloroform (unless otherwise noted) with a Rudolph polarimeter 139 and are approximated to the nearest degree. Nuclear magnetic resonance spectra were obtained at 60 Mc on a Varian Associates A-60 or A-60A recording spectrometer in deuteriochloroform (unless otherwise noted) using tetramethylsilane as the internal standard and were electronically integrated. Analytical gas chromatography was executed on an F & M Scientific Corp. Model 700 gas chromatograph. Thin layer chromato-

in diethylene glycol (150 ml) was prepared by gentle warming in an oil bath. After cooling, 9β,19-cyclo-5α-pregnane-3,11,20-trione 3,20-diethylene ketal (V, 2.5 g) and anhydrous hydrazine (16.8 ml, dried by refluxing over potassium hydroxide and distilling) were added. The reaction mixture was heated under reflux at 170° (internal) for 2 hr. The temperature was raised to 210° (internal) and the condenser was removed and then replaced after 1 hr. The reaction mixture was kept at the same temperature for 2 more hr. Cooling was followed by addition of water (400 ml) and extraction with three 150-ml portions of chloroform. The organic layer was back-washed with three 100-ml portions of water, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to yield a yellowish gum (2.7 g).

Chromatography of the Wolff–Kishner Product. A. Column Chromatography. The gum obtained as described above was dissolved in Skellysolve B (5 ml) and was added to a column of Woelm grade II neutral alumina (75 g). Elution with Skellysolve B (450 ml) gave isomer IX (0.58 g) in crude crystalline form. The second fraction (1.3 g), obtained with Skellysolve B (600 ml), was a mixture of IX and X. The last fraction (0.22 g), obtained by eluting with benzene (200 ml), proved to be crude isomer X. Recrystallization of the first fraction from methanol–ether gave pure isomer IX as colorless plates (0.245 g): mp 109–110°; $[\alpha]^{25}D$ −25° (*c* 0.29); $[\alpha]^{24}D$ −25° (*c* 0.106, methanol); $\lambda_{max}^{CHCl_3}$ 10.54 μ (ketal); nmr (CCl_4), τ 9.27 (3 H, s), 8.81 (3 H, s), 6.15 (8 H, s), 4.89 (1 H, half-width = 8 cps).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.79; H, 9.66.

The third fraction crystallized from methanol–ether to yield isomer X (0.063 g): mp 124–126°; $[\alpha]^{25}D$ −54° (*c* 0.82); λ_{max} 10.51 μ; nmr (CCl_4), τ 9.27 (3 H, s), 8.80 (3 H, s), 6.17 (8 H, s), 4.83 (1 H, half-width = 8.5 cps).

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.67; H, 9.46.

An improved column chromatographic procedure developed later utilized a 1:150 ratio of compound to Woelm neutral alumina (deactivated with water, 2.5% v/w) and benzene–Skellysolve B (1:9) as eluent.

B. Thick Layer Chromatography. A representative experiment involved the application of the mixture of IX and X (approximately 50 mg) in chloroform to a plate (0.5 mm) of Brinkman aluminum oxide G. Benzene was used for development and ultraviolet light was used for the detection of the band. The upper and lower parts of the band were cut off, extracted with chloroform, and crystallized from methanol–ether. The middle part was rechromatographed in the same manner. This method yielded the products in the proportion of 60% IX and 40% X.

C. Gas Chromatography. A stainless steel column (4 ft × 0.25 in.) was prepared as follows. Chromosorb W (ABS 70–80 mesh, 20 g) was stirred on a steam bath with 1% solution of fluorosilicone (QF 10065) in acetone (100 ml) until a pasty consistency was obtained. Stirring was continued for 10 more min at room temperature, after which the mixture was dried in an oven at 80° for 10 hr. The column was packed by applying vacuum (20 mm) at one end and filled from the other end with small portions with tapping. The temperatures of the various parts were as follows: injection point, 300°; detector, 300°; column, 250°. Nitrogen was used as the carrier gas, at a rate of 1.0 ml/sec. The mixture was injected in 1% solution in chloroform (2 μl). Two peaks appeared, indicating approximately the same composition as the thick layer chromatographic separation.

9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-Pregnene-3,20-dione 3-Monoethylene Ketal (XI). The diketal, 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-pregnene-3,20-dione diethylene ketal (IX, 130 mg), in a mixture of benzene–ether (1:1, 12 ml) was treated with freshly distilled boron trifluoride ethereate (0.4 ml) and the reaction mixture was left at room temperature for 4 hr. Dilution with ether (25 ml) was followed by shaking with 5% sodium bicarbonate solution (10 ml). The organic layer was washed with three 10-ml portions of water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. Thin layer chromatographic analysis with benzene showed the residue (110 mg) to be a mixture of the starting material and the product. Preparative thick layer chromatography afforded the starting material (32 mg) and the product (XI, 65 mg) as colorless prisms from acetone–Skellysolve B; mp 134–136°; $[\alpha]^{30}D$ +36° (*c* 0.70); $\lambda_{max}^{CCl_4}$ 5.90 μ; nmr (CCl_4), τ 9.37 (3 H, s), 7.98 (3 H, s), 6.13 (4 H, s), 4.86 (1 H, m).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 76.62; H, 9.77.

9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-Pregnene-3,20-dione 3-Monoethylene Ketal (XII). The diketal, 9(10→19)*abeo*-$\Delta^{(9\,11)}$-5α,10α-pregnene-3,20-dione diethylene ketal (X, 23 mg), was treated in the same manner as above to give, after preparative thick layer chromatography, the starting material (5 mg) and the product XII (11.5 mg), as colorless prisms from Skellysolve B; mp 90–92°; $[\alpha]^{29}D$ +35° (*c* 0.10); $\lambda_{max}^{CHCl_3}$ 5.90 μ; nmr (CCl_4), τ 9.37 (3 H, s), 7.87 (3 H, s), 6.0 (4 H, s), 4.65 (1 H, m).

Anal. Calcd for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.24; H, 9.62.

9(10→19)*abeo*-5α,10β-Pregnane-3,20-dione 3,20-Diethylene Ketal (XIII). 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-Pregnene-3,20-dione diethylene ketal (IX, 37 mg) was hydrogenated with platinum oxide (20 mg) in 95% ethanol (4 ml) for 18 hr. The solution was filtered through Celite and evaporated to leave a white solid (41 mg), mp 112–113°. Recrystallization from ethyl acetate–methanol gave pure XIII (26 mg): mp 121–122°; λ_{max} 10.55 μ (ketal); nmr, τ 9.21 (3 H, s), 8.72 (3 H, s), 6.07 (4 H, s).

Anal. Calcd for $C_{25}H_{40}O_4$: C, 74.22; H, 9 97. Found: C, 74.49; H, 10.34.

9(10→19)*abeo*-5α,10α-Pregnane-3,20-dione 3-Monoethylene Ketal (XV). 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10β-Pregnene-3,20-dione 3,20-diethylene ketal (IX, 143 mg) was hydrogenated as described above. An oven-dried column was charged with Woelm neutral alumina (10 g, undeactivated) and Skellysolve B. The crude hydrogenation product (143 mg) in Skellysolve B was absorbed onto the alumina and allowed to stand at room temperature for 7 hr. Then the column was eluted with chloroform–benzene (1:10) to give a gum (113 mg). This gum was chromatographed on Woelm neutral alumina (15 g, deactivated with water, 3% v/w). Elution with Skellysolve B (1 l.) gave a solid residue (20 mg) which showed spectral properties corresponding to diethylene ketal derivatives. Further elution with Skellysolve B (320 ml) gave a mixture (33 mg) containing some of the desired XV. The next volume (240 ml) of Skellysolve B eluted XV (17 mg); crystallization from methanol gave pure XV (8 mg): mp 136–137°; $[\alpha]^{25}D$ +49° (*c* 0.0305, methanol); λ_{max} 5.87, 5.89 (carbonyl), and 10.58 μ (ketal); nmr, τ 9.37 (3 H, s), 7.89 (3 H, s), 6.07 (4 H, s).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07 Found: C, 77.15; H, 10.05.

9(10→19)*abeo*-5α,10β-Pregnane-3,20-dione (XVII). a. A solution of 9(10→19)*abeo*-5α,10β-pregnane-3,20 dione 3,20-diethylene ketal (XIII, 20 mg) and *p*-toluenesulfonic acid monohydrate (1 mg) in acetone (1 ml) was allowed to stand for 48 hr at room temperature. The acetone was evaporated with a stream of nitrogen, chloroform and water were added, and the layers were separated. The aqueous layer was reextracted with chloroform and the combined chloroform layers were dried over anhydrous sodium sulfate, filtered, and evaporated to yield a faint yellow gum (17.4 mg). Crystallization from Skellysolve C–ether gave pure XXVII (5.8 mg); mp 98.5–100°; $[\alpha]^{25}D$ +87° (*c* 0.0196, methanol); λ_{max} 5.83 and 5.87 μ; nmr, τ 9.35 (3 H, s), 7.88 (3 H, s).

Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.67; H, 10.28.

b. A solution of 9(10→19)*abeo*-5α,10β-pregnane-3,20-dione 3-monoethylene ketal (XV, 8.2 mg) and *p*-toluenesulfonic acid monohydrate (1.4 mg) in water (0.3 ml) and acetone (2 ml) was refluxed for 3 25 hr. After cooling to room temperature most of the acetone was evaporated with a stream of nitrogen. Water and benzene were added, the layers were separated, and the aqueous layer was extracted again with benzene. The combined organic layers were dried over anhydrous magnesium sulfate, filtered, and evaporated to yield a semicrystalline product (8 mg). The product was chromatographed on Woelm neutral alumina (1 g, deactivated with water, 2% v/w) using Skellysolve B–benzene (1:1) as the eluent. One fraction (3 mg) was crystallized from Skellysolve B to give XVII, mp 100–101°, which was shown by mixture melting point and thin layer chromatography to be the same as the compound prepared by method a.

9(10→19)*abeo*-5α,10β-Pregnane-3,20-dione 3-Monoethylene Ketal (XVI). 9(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-Pregnene-3,20-dione 3,20-diethylene ketal (X, 40.4 mg) was hydrogenated with platinum oxide (25 mg) in 95% ethanol (3.7 ml) for 19 hr. After filtering off the catalyst, the solution was evaporated to dryness to provide a colorless gum (39 mg). The product (72 mg) of two such reactions

graphic plates were sprayed with 3% ceric sulfate solution in 3 *N* sulfuric acid and heated in an oven. Optical rotatory dispersion curves were determined in methanol on a Cary 60 recording spectrophotometer. The authors are grateful to Mr. Joseph Alicino (Metuchen, N. J.) and Mr. Arthur Spang (Ann Arbor, Mich.) for microanalyses.

was dissolved in benzene–Skellysolve B (1:1), adsorbed onto a column of Woelm neutral alumina (6 g, undeactivated), and left on the column for 11 hr. Elution of the column with chloroform (300 ml) gave crude monoketone XVI (59 mg). The crude material was then chromatographed on Woelm neutral alumina (15 g, deactivated with water, 4% v/w). Elution with Skellysolve B–benzene (4:1, 300 ml) gave a mixture (3 mg) with spectral properties corresponding to diethylene ketal derivatives. Further elution with Skellysolve B–benzene (3:2, 100 ml) gave the desired monoketone XVI (20 mg). Crystallization of this material from Skellysolve C gave pure XVI: mp 118–120°; $[\alpha]^{25}D$ +72° (*c* 0.032, methanol); λ_{max} 5.86 μ; nmr, τ 9.38 (3 H, s), 7.90 (3 H, s), 6.05 (4 H, s).

Anal. Calcd for $C_{23}H_{36}O_3$: C, 76.62; H, 10.06. Found: C, 76.38; H, 10.46.

The next volume (120 ml) of Skellysolve B–benzene (3:2) yielded three-spot material (11 mg). Finally, elution with Skellysolve B–benzene (3:2, 125 ml) and with benzene (100 ml) gave some diketone (22 mg).

9(10→19)*abeo*-5α,10α-Pregnane-3,20-dione (XVIII). a. 9-(10→19)*abeo*-$\Delta^{9(11)}$-5α,10α-Pregnene-3,10-dione 3,20-diethylene ketal (X, 70 mg) was hydrogenated as described above to yield crude product (68 mg). A solution of this product and *p*-toluenesulfonic acid monohydrate (5 mg) in water (1 ml) and acetone (4 ml) was refluxed for 3.5 hr and then left at room temperature overnight. The acetone was removed under reduced pressure at room temperature and the remaining residue was treated with water (5 ml) and benzene (10 ml). The organic layer was separated and the aqueous layer was extracted twice with chloroform–benzene (1:2, 10 ml). The combined organic layer was washed with saturated salt solution and dried over anhydrous magnesium sulfate. After filtration the solution was evaporated to dryness to leave a colorless oil (50 mg). This oil was combined with the diketone (22 mg) obtained during preparation of the monoketone. The combined material was chromatographed on Woelm neutral alumina (15 g, deactivated with water, 3.5% v/w). Elution with Skellysolve B–benzene (1:1, 220 ml) gave a mixture (30 mg) containing some of the desired diketone. Further elution with Skellysolve B–benzene (1:2, 90 ml) furnished crystalline XVIII (6 mg). Recrystallization from *n*-hexane provided pure XVIII (4 mg): mp 149–150°; $[\alpha]^{26}D$ +94° (*c* 0.015, methanol); λ_{max} 5.88 μ; nmr, τ 9.38 (3 H, s), 7.90 (3 H, s).

Anal. Calcd for $C_{21}H_{32}O_2$: C, 79.70; H, 10.19. Found: C, 79.51; H, 10.01.

b. A solution of 9(10→19)*abeo*-5α,10α-pregnane-3,20-dione 3-monoethylene ketal (XVI, 10 mg) and *p*-toluenesulfonic acid monohydrate (2 mg) in acetone (3 ml) and water (0.3 ml) was refluxed for 3 hr. Acetone was removed by evaporation under reduced pressure at room temperature and the residue was treated with water (3 ml) and extracted with three 5-ml portions of benzene. The benzene extract was washed once with water and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure to leave a gray residue (6 mg). This residue on crystallization from Skellysolve C provided diketone XVIII (2 mg), mp 147–150°. The melting point was not depressed upon admixture with the diketone prepared by method a, and the thin layer chromatographic behavior of the materials were identical.

Manifestations of the Tertiary Structures of Proteins in High-Frequency Nuclear Magnetic Resonance

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Abstract: Manifestations in high-frequency (220 Mcps) proton magnetic resonance spectroscopy of the conformational differences that exist between the native and denatured forms of ribonuclease, lysozyme, and cytochrome *c* are described. High-field resonances in the 0.7- to −1.0-ppm range (relative to the internal standard 2,2-dimethyl-2-silapentane-5-sulfonate) are exhibited by lysozyme and cytochrome *c only* in the folded, native conformations. The unusual positions of these resonances are attributed to high-field ring current shifts that are induced in the highly shielded C–H protons of such residues as leucine, isoleucine, valine, lysine, methionine, arginine, threonine, and alanine whose side chains are proximal to the faces of the aromatic groups of the histidine, phenylalanine, tyrosine, and tryptophan residues in the proteins' folded conformations. Additional, even larger diamagnetic shifts (in the −2.0- to −3.8-ppm range) are observed in the pmr spectra of the oxidized and reduced forms of folded cytochrome *c* that are believed to derive from side chains of component amino acids that experience the large ring current field associated with the extensively conjugated porphyrin ring. Pmr spectral differences between ferricytochrome *c* and ferrocytochrome *c* strongly suggest that important conformational dissimilarities exist between the oxidized and reduced forms of the protein that are due to differences in side-chain coordination at the heme iron. High-field resonances characteristic of the folded conformations are also observed for native myoglobin and hemoglobin. One of these that occurs at −3.7 ppm in the spectrum of myoglobin has a resonance width of 110 cps. This width is attributed to paramagnetic relaxation by the high-spin heme iron. Conversion of myoglobin to the low-spin form as the result of coordination of CN^- to the heme iron leads to the appearance of two sharp resonances in the −3.0- to −3.3-ppm region of the spectrum.

There have been a number of studies of proteins in solution by proton magnetic resonance (pmr) spectroscopy.[1-11] Structural information derivable from such studies has been severely limited by the overlap of chemical shifts of the very large number of structurally

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