

Acid-Catalyzed Rearrangement of 20 α -Ethynylpregn-5-ene-3 β ,20 β -diol 3-Acetate¹

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The unimolecular acid-catalyzed reaction of 20 α -ethynylpregn-5-ene-3 β ,20 β -diol 3-acetate (I) has been shown to give both Wagner–Meerwein and Rupe rearrangement products. The structural assignment of the D-homosteroid II, resulting from the Wagner–Meerwein rearrangement, is based on spectroscopic evidence and mechanistic considerations. The Rupe rearrangement has been shown to give two α,β -unsaturated methyl ketones III and IV. These structures have been established by chemical degradation as well as by synthesis from the α,β -unsaturated nitriles XVIII and XVII, respectively. The *cis*–*trans* geometry of these nitriles and ketones is based on their n.m.r. characteristics. The conformations of the ketones have been determined by their ultraviolet and infrared absorption characteristics. The reaction of the ethynyl carbinol I with phosphorus oxychloride and pyridine, and also with thionyl chloride in dimethylformamide, has been shown to give the enyne V as the major reaction product, along with small amounts of the enynes VI and VII and the chloroallene XIX. The structures of these compounds have been determined with the help of their spectroscopic properties.

Ethynylcarbinols, on treatment with acids, are known to undergo isomeric rearrangements to give α,β -unsaturated carbonyl compounds. These rearrangements, known as Meyer–Schuster and Rupe rearrangements, follow the unimolecular mechanism and are believed to take place through the intermediate formation of a mesomeric carbonium ion which may react, depending upon its structure, in either of the two ways shown in Chart I.² These reactions are driven forward by the irreversible processes involved in the formation of the carbonyl compounds from the intermediate carbonium ions. The recent work of Smissman, *et al.*,³ on the rearrangement of *t*-butylphenylethynylcarbinol and of Jacques and his collaborators⁴ on the rearrangement of 17 α -ethynyl-17 β -hydroxy steroids shows that in appropriately substituted systems Wagner–Meerwein rearrangement of the intermediate carbonium ion may compete with these irreversible processes.

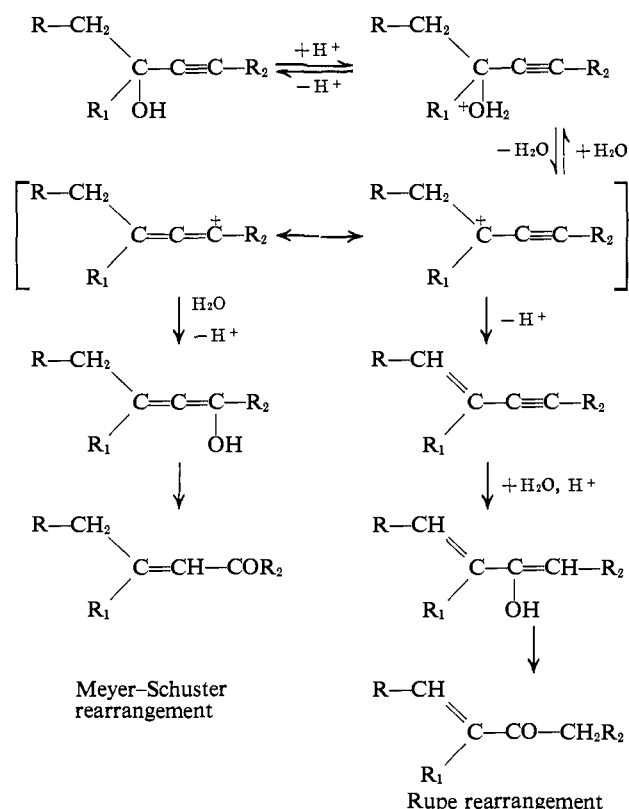
(1) (a) Presented, in part, at the 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 6–10, 1964. (b) This investigation was supported by Grants AM-03419 and AM-07280 of the National Institute of Arthritis and Metabolic Diseases. (c) All the n.m.r. spectra were obtained with a Varian Associates Model V-4300 B spectrometer. The samples were run as *ca.* 20% deuteriochloroform solutions containing 1% v/v. tetramethylsilane as an internal reference, and the n.m.r. signals are reported in c.p.s. at 60 Mc.

(2) P. B. D. de la Mare in "Molecular Rearrangements", Part I, P. de Mayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1963, p. 27.

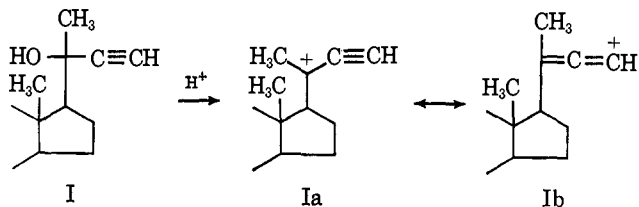
(3) E. E. Smissman, R. H. Johnsen, A. W. Carlson, and B. F. Aycok, *J. Am. Chem. Soc.*, **78**, 3395 (1956).

(4) M. Dvolaitzky, A. M. Giroud, and J. Jacques, *Bull. soc. chim. France*, **62** (1963); C. Ouannes, M. Dvolaitzky, and J. Jacques, *ibid.*, **776** (1964).

Chart I



Our interest in the synthesis of $\Delta^{17(20)}$ -22-keto steroids led us to investigate the course of the acid-catalyzed rearrangement of a 20-ethynyl-20-hydroxy steroid I, since the Rupe rearrangement would give such a steroid. Alternatively, however, the intermediate propargyl cation⁵ Ia (which may be in res-



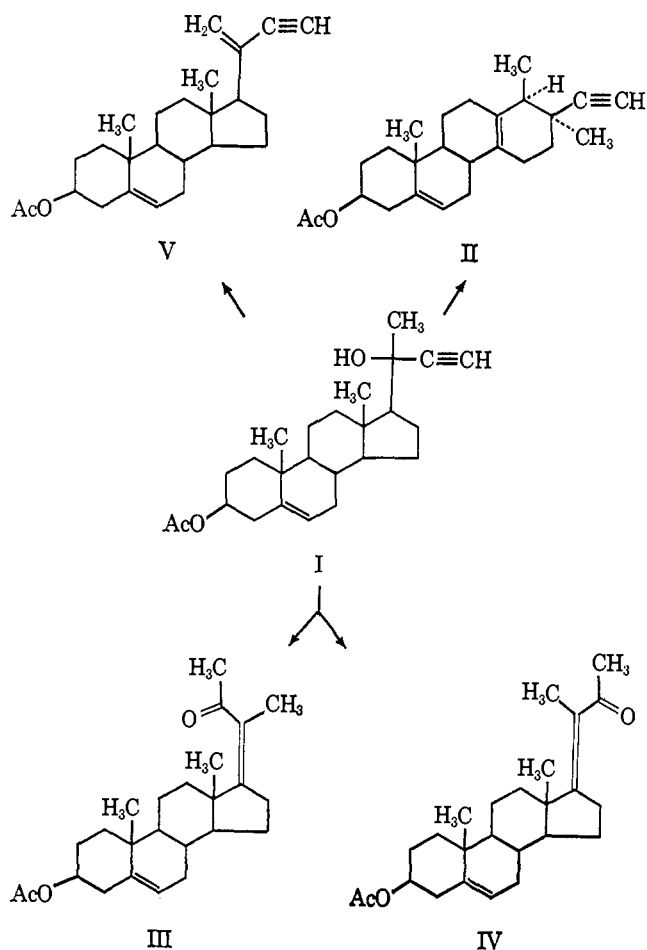
onance with the allenic cation Ib) may undergo a Wagner–Meerwein rearrangement to give a D-homosteroid in order to relieve the steric strain of the *trans*-hydrindan system. As a matter of fact, D-homannulation has been known to be a general reaction of steroids which react through the intermediate formation of a carbonium ion at C-20.⁶

(5) Recently, H. G. Richey, Jr., J. Ch. Phillips, and L. E. Rennick, *J. Am. Chem. Soc.*, **87**, 1381 (1965), have made the first direct observation of such a cation by treatment of an ethynylcarbinol with sulfuric acid.

(6) N. L. Wendler in "Molecular Rearrangements," Part 2, P. de Mayo, Ed., Interscience Publishers, Inc., New York, N. Y., 1964, p. 1019.

The compound chosen for our study was 20 α -ethynylpregn-5-ene-3 β ,20 β -diol 3-acetate⁷ (I) which was prepared by ethynylation of 3 β -hydroxypregn-5-en-20-one acetate. We found that when I was treated with 97% formic acid or acetic acid containing a catalytic amount of sulfuric acid for 3–5 min. on a steam bath, it gave three major products (see Chart II): (a) D-

Chart II



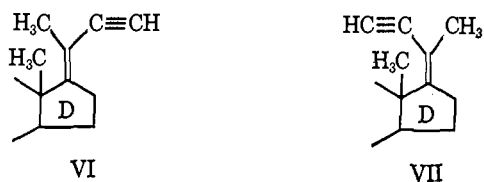
homosteroid II, m.p. 141–142°, which is a nonconjugated enyne, and two isomeric α,β -unsaturated methyl ketones III, m.p. 176–178°, and IV, m.p. 119–121°, which constitute a pair of $\Delta^{17(20)}$ -geometrical isomers. In addition to these, a very small amount of a conjugated enyne V, m.p. 125–127°, was also obtained as a minor product. The yields of pure II, III, IV, and V were about 25, 25, 10, and 1%, respectively. When the reaction was carried out at room temperature, it took about 24 hr. for completion, and the yields of the ketones III and IV were slightly increased (to about 40 and 15%, respectively) but the yield of the D-homosteroid II remained practically unchanged.

The isolation of the different products from the reaction mixture was carried out by chromatographic separation on an alumina column as described in the Experimental section.

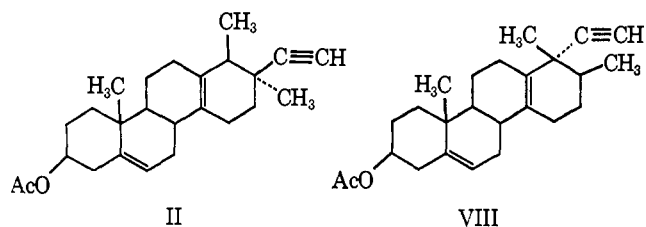
Structure of the D-Homosteroid II. The assignment of the D-homo structure II is based on the elemental analysis and the spectral characteristics of the compound. The infrared absorption peaks at 3.05 and 4.75 μ showed the presence of an ethynyl group, and

(7) F. Sondheimer, N. Danieli, and Y. Mazur, *J. Org. Chem.*, **24**, 1278 (1959).

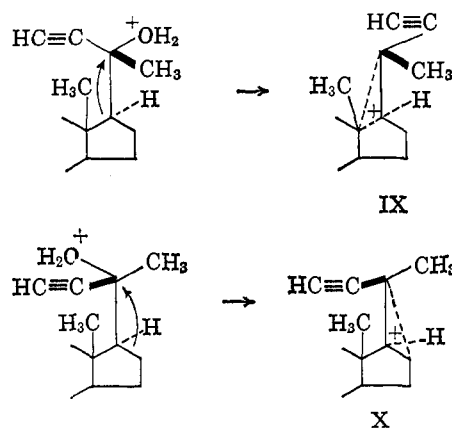
the lack of any characteristic absorption in the ultra-violet showed the absence of any conjugated enyne system in the molecule. The structures V, VI, and VII were, therefore, ruled out. The n.m.r. spec-



trum of the compound showed the absence of any peak due to vinylic protons other than the one at C-6, the presence of three tertiary methyl peaks at 60.5, 70, and 121 c.p.s., and a secondary methyl peak (symmetrical doublet, $J = 7$ c.p.s.) centered at 60 c.p.s. The peak at 60.5 c.p.s. may be assigned to the 19-methyl and the peak at 121 c.p.s. to the methyl of the 3 β -acetate function. The peak due to the ethynyl hydrogen atom was shifted upfield⁸ and overlapped with the peak due to the methyl group of the acetate function. When the acetate group was removed by hydrolysis, the ethynyl hydrogen peak appeared at 122 c.p.s. These spectroscopic characteristics can be explained by assigning a D-homosteroid structure (II or VIII) having a tertiary methyl, an ethynyl, and a secondary methyl group on the D ring, and a double



bond in the 13,14 position. Between the two alternative structures II and VIII, the former is favored from a consideration of the steric interactions⁹ involved in the transition state leading to the movement of the 13–17 or the 16–17 bond. Owing to the linear nature of the ethynyl group, the steric interaction involved in its

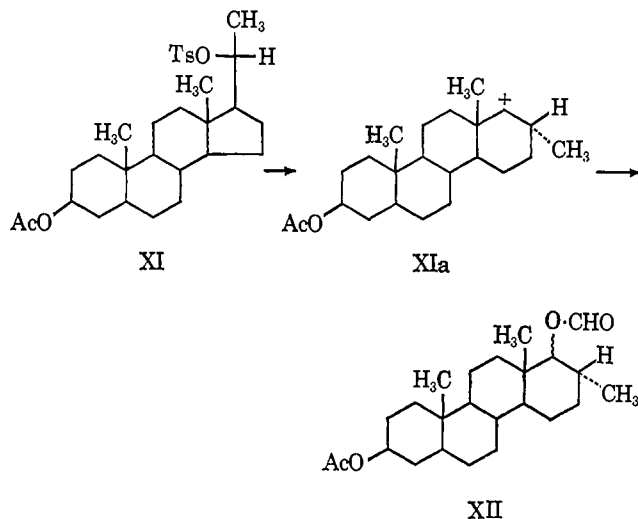


eclipsing with the groups at C-13 in the structure X should be less than the interaction involved in the

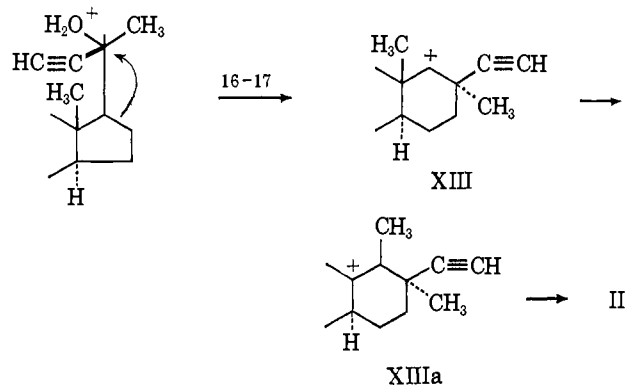
(8) S. J. Cristol and J. K. Harrington, *ibid.*, **28**, 1413 (1963), have observed a similar upfield shift of the peak due to the ethynyl hydrogen of 4-ethynylcyclopentene.

(9) Although electronic factors would favor 13–17 bond migration, in case of competition with steric factors, the latter usually prevail: cf. D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling, and G. Roberts, *J. Am. Chem. Soc.*, **77**, 6585 (1955).

eclipsing of the 21-methyl and 16-methylene groups in the structure IX. It is, therefore, reasonable to assume that the D-homoannulation has taken place by the movement of the 16-17 bond as in the case of the solvolytic rearrangement¹⁰ of the tosylate XI to give XII. The rearrangement of I to give II may



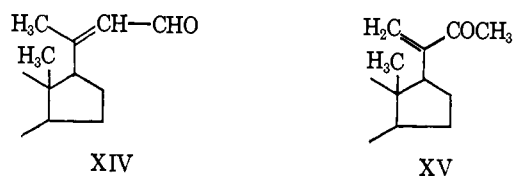
take place either in a concerted manner or in a step-wise process *via* XIII and XIIIa.



Structure of the Unsaturated Ketones III and IV. The elemental analysis showed that both the compounds III and IV are isomeric with the ethynyl alcohol I. The infrared spectrum of III (m.p. 176-178°) showed a carbonyl band at 1685 and a weak C=C band at 1640 cm^{-1} . The spectrum of IV (m.p. 119-121°) also showed a carbonyl band (1670 cm^{-1}) and a strong C=C band (1590 cm^{-1}). III had an ultraviolet absorption maximum at 249 $\text{m}\mu$ (ϵ 3000) and IV at 253 $\text{m}\mu$ (ϵ 9000). One may conclude from the above data that both III and IV are α,β -unsaturated carbonyl compounds. The considerably lower value of the molecular extinction and the shifting of the position of the ultraviolet absorption maximum and of the carbonyl peak in the infrared to lower wave length in the case of III may be explained by assigning a nonplanar conformation to III so that there is less conjugation of the C=O group and C=C double bond.

The alternative structures XIV (resulting from a Meyer-Schuster rearrangement) and XV were ruled out from a consideration of the n.m.r. spectra of the

(10) H. Hirschmann and J. S. Williams, *J. Biol. Chem.*, **238**, 2305 (1963).



rearrangement products which showed the absence of any aldehydic and vinylic proton other than the one at C-6. Besides, a synthetic sample of XV, prepared by catalytic hydration of the triple bond of V, was found to be different from either of the rearrangement products.

The positions of the n.m.r. peaks (in cycles per second) due to the various protons of III and IV are shown in Table I. Both III and IV are found to have one vinylic proton (C-6), two tertiary methyl groups (18 and 19 methyls), one methyl group adjacent to a carbonyl group (-COCH₃ group), one methyl group on an unsaturated carbon atom, and a methyl group of the 3 β -acetate function.

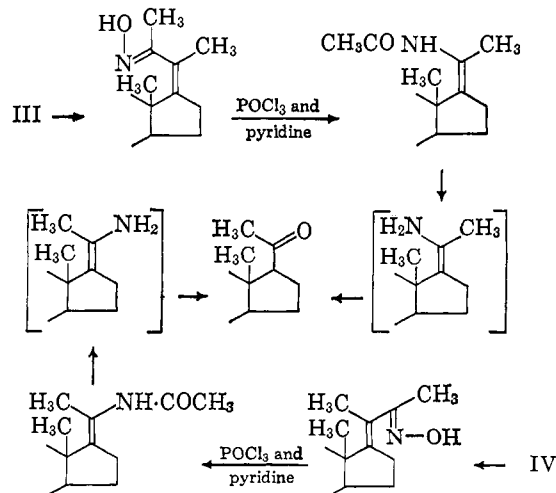
Table I

	6-H	3 α -H	-COMe	3 β -OAc	=C-Me	19-Me	18-Me
III	323 ^a	278	135	122.5	106 ^b	61.5	58
IV	324 ^a	278	135	122.5	119 ^c	62.5	56

^a Doublet, $J = 4$ c.p.s. ^b Broad. ^c Triplet, $J = 1.5$ c.p.s.

The following chemical degradation showed that the D ring of both III and IV is five membered and thus ruled out any D-homo structure. When the oximes of III and IV were treated with phosphorus oxychloride in pyridine, they underwent Beckmann rearrangement to give the corresponding unsaturated amides (identified by infrared spectra) which on hydrolysis with acids gave 3 β -hydroxypregn-5-en-20-one acetate. These results can be rationalized by assigning $\Delta^{17(20)}$ -22-keto structures to III and IV. The formation of 3 β -hydroxypregn-5-en-20-one acetate from III and IV may be explained as shown in Chart III.

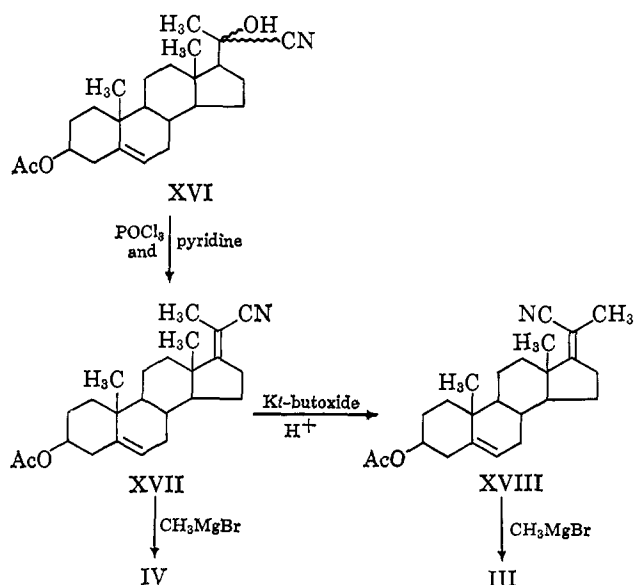
Chart III



Since $\Delta^{17(20)}$ -steroids would exhibit geometrical isomerism around the 17-20 double bond, one of the ketones has the *cis* and the other *trans* geometry.

Synthesis of III and IV. The $\Delta^{17(20)}$ structures III and IV were confirmed by their synthesis from the cor-

responding α,β -unsaturated nitriles XVIII and XVII, respectively. The 1:2 addition of methylmagnesium bromide to XVIII and XVII gave the corresponding ketimines, which on hydrolysis with acids gave the ketones III and IV, respectively. XVII was prepared



by dehydrating the cyanohydrin XVI with phosphorus oxychloride and pyridine. This dehydration had been described by Sarett¹¹ who assigned a $\Delta^{17(20)}$ structure to the dehydration product in analogy with 11-keto-21-hydroxy systems. In view of the results of dehydration of other 20-hydroxy steroids, we felt it necessary to establish the position of the double bond unequivocally, and to determine the stereochemistry of the dehydration product in the case of a $\Delta^{17(20)}$ structure.

The 17–20 position of the double bond was indicated by the ultraviolet spectrum of the nitrile which showed a maximum at 222 $m\mu$ (ϵ 14,800). This is characteristic¹² of α,β,β -trisubstituted α,β -unsaturated nitriles. The nitrile with the double bond in the 20–21 position would have the maximum at 205–208 $m\mu$.¹³ The $\Delta^{17(20)}$ structure was confirmed by the n.m.r. spectrum of the nitrile which showed the presence of a methyl group on an unsaturated carbon (21-methyl) and the absence of any vinylic proton other than the one at C-6.

When the above nitrile (m.p. 176–177°) was treated with potassium *t*-butoxide in dimethyl sulfoxide it gave, after acidification with acetic acid and reacetylation of the 3-OH group, an isomeric nitrile (m.p. 212–214°) which had the same ultraviolet characteristics as the lower melting one, and its n.m.r. spectrum also showed the presence of the 21-methyl group and absence of any 21-vinylic proton. It may, therefore, be concluded that the higher melting nitrile is a geometrical isomer of, and more stable than, the lower melting nitrile. It should, therefore, have the *trans* structure XVIII because in that structure there is less steric interaction between the 12-methylene group and the nitrile group, owing to its linear nature. In the *cis* structure XVII, there is considerable interaction between the 12-methylene and the 21-methyl group

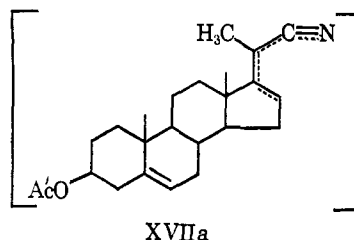
(11) L. H. Sarett, *J. Am. Chem. Soc.*, **70**, 1454 (1948).

(12) O. H. Wheeler, *J. Org. Chem.*, **26**, 4755 (1961).

(13) R. Heilmann, J. M. Bonnier, and G. de Gaudemais', *Compt. rend.*, **244**, 1787 (1957).

and this should, therefore, represent the less stable isomer (m.p. 176–177°).¹⁴

The above base-catalyzed isomerization may be visualized as taking place through the mesomeric anion XVIIa which, on equilibration, gives the more stable isomer XVIII.



This stereochemical assignment of *cis* and *trans* structures is also supported by the n.m.r. spectra of the nitriles. The homoallylic coupling constant of the spin-spin splitting of the 21-methyl protons with the 16-methylene protons is expected to be slightly higher in the structure XVII (in which the 21-methyl and the 16-methylene groups are *trans* oriented) than in the structure XVIII, where they are *cis* oriented. The homoallylic coupling constants so far reported¹⁵ lie in the range of 0–1.6 c.p.s. and the transoid coupling constants are usually larger by 0.3–0.5 c.p.s. The peak due to the 21-methyl protons of the lower melting nitrile appeared as a triplet having $J = 1.6$ c.p.s., whereas that of the higher melting nitrile showed unresolved coupling, indicating thereby lower values for J . The lower melting nitrile should, therefore, have the structure XVII, and the higher melting nitrile, XVIII. The position of the 21-methyl peak of the two nitriles in their n.m.r. spectra is also in agreement with the assigned structures. In the *cis* structure XVII, the steric interaction between the 18-methyl and the 21-methyl groups would produce a downfield shift¹⁶ of these protons, whereas in the *trans* structure XVIII, only the 18-methyl protons would be deshielded by the nitrile group. The resonance signal of the 21-methyl protons of the *cis* structure XVII would, therefore, appear at a lower field than the signal of the 21-methyl protons of the *trans* structure XVIII. Since the 21-methyl peak of the lower melting nitrile appeared as a triplet centered at 115.5 and that of the higher melting nitrile appeared at 108.5 c.p.s., the lower melting nitrile should have the structure XVII.

Stereochemistry of the Ketones III and IV. On the basis of above syntheses, the higher melting ketone (176–178°) may be assigned the *trans* structure III and the lower melting ketone (119–121°) may be assigned the *cis* structure IV. This assignment is confirmed by the position of the 21-methyl peak in the n.m.r. spectra of the ketones and the homoallylic

(14) Huang-Minlon, R. Tull, and J. Babcock, *J. Am. Chem. Soc.*, **76**, 2396 (1954), have effected this type of geometrical isomerization in a closely related system.

(15) Cf. N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p. 110, and references therein.

(16) Recently, B. J. Magerlein, R. D. Birkenmeyer, and F. Kagan, *J. Org. Chem.*, **28**, 3474 (1963), have observed the same deshielding influence on the C-18 protons by *cis*- $\Delta^{17(20)}$ -21-hydroxy compounds relative to the *trans* isomers, and W. R. Benn and R. M. Dodson, *ibid.*, **29**, 1142 (1964), have observed the deshielding influence on both the C-18 and C-21 protons by the *cis*-16 α -acetoxy- $\Delta^{17(20)}$ -ethylidene systems relative to the *trans* systems.

coupling constants of the spin-spin splitting of the 21 methyl protons with the 16-methylene protons. The peak due to the 21-methyl protons of the lower melting ketone IV appeared as a triplet centered at 119 c.p.s. with the coupling constant of 1.5 c.p.s., whereas the peak of the higher melting ketone appeared slightly broadened at 106 c.p.s.

An examination of the Dreiding model reveals that both the *s-cis* and *s-trans* conformations of the ketone III are sterically unfavorable because of the interference of the 12-methylene group with the CO group (in the case of *s-cis*) and with the methyl group (with the *s-trans*) of the $-\text{COCH}_3$ group. The ketone III has, therefore, a nonplanar conformation as suggested before. If one uses the equation $\epsilon/\epsilon^0 = \cos^2(\theta_1 - \theta_2)$ developed by Braude and Timmons¹⁷ and recently revised by Forbes and Mueller,¹⁸ the interplanar angle may be calculated to be about 60°.

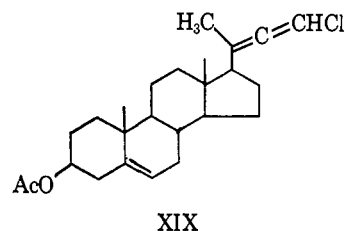
Since the ketones with an enforced *s-cis* conformation have ϵ -values of about 5000–10,000,¹⁹ the lower melting ketone IV with ϵ -value of 9000 may be assumed to have the *s-cis* conformation. This conclusion is also supported by the infrared characteristics of IV. The C=O and C=C absorption bands in the infrared spectrum of IV are of comparable intensities and they are separated by 80 cm^{-1} . According to Erskine and Waight,²⁰ the ratio of the integrated band intensities of the carbonyl and double bond stretching vibrations lies between 0.6 and 3.5 for *s-cis* conformations and is greater than 6 for *s-trans* forms. The C=O and C=C bands are usually separated by more than 75 cm^{-1} in the *s-cis* form.²¹ One may conclude from these considerations that IV has the *s-cis* conformation. An inspection of the Dreiding model of IV shows that in the *s-trans* conformation there is considerable interaction between the 16-methylene group and the methyl group of the $-\text{COCH}_3$ function and, therefore, IV takes the *s-cis* conformation.

Structure of the Conjugated Enyne V, M.p. 125–127°. The structural assignment of the conjugated enyne V is based on its ultraviolet, infrared, and n.m.r. characteristics. The ultraviolet absorption spectrum showed a maximum at 223 with a shoulder at 228 $\text{m}\mu$, which is characteristic²² of monosubstituted vinylacetylenes. The presence of the terminal methylene group (21-methylene) was confirmed by the presence, in the infrared spectrum, of a strong band at 915 and a weak band at 1830 cm^{-1} (overtone of 915 cm^{-1}). Moreover, the n.m.r. spectrum showed the presence of three vinylic protons, including one at C-6.

Synthesis of the Conjugated Enynes V, VI, and VII. In an earlier paper, Sondheimer, *et al.*,⁷ reported that the dehydration of I with phosphorus oxychloride and pyridine gave a conjugated enyne which had an ultraviolet absorption maximum at 223 $\text{m}\mu$, an infrared band at 11.14 μ (895 cm^{-1}), and a melting point of 103–105°. The structure V was assigned to this com-

pound. Since the melting point and the position of the infrared bands of our compound were slightly different from those reported by these workers, we investigated this reaction and found that although the major product was V, small amounts of the enynes VI and VII and the chloroallene XIX were also formed. An attempt to dehydrate with thionyl chloride in dimethylformamide also led to a similar mixture. Spectroscopically pure samples of V, VI, and VII were obtained through repeated chromatography and fractional crystallizations. The small differences in the physical properties of our compound V and the compound obtained by the earlier workers may be due to the presence of a small amount of the chloroallene in their sample, since we found that an impure sample of V had a broad band at 895–915 cm^{-1} and the small overtone band at 1830 cm^{-1} could not be observed. When the last trace of the contaminating chloroallene was removed, a sharp band appeared at 915 with small shoulders at 900 and 895 cm^{-1} , and the overtone band of 1830 cm^{-1} could be observed.

We could not prepare an analytically pure sample of the chloroallene XIX which was possibly mixed with a small amount of the enyne V. However, the presence of a strong band at 1950 (allenic double bond) and several bands in the region of 750–700 cm^{-1} (C–Cl bands) in the infrared spectrum suggested the structure XIX. This is also supported by the n.m.r. spectrum which showed the presence of a methyl group (21-methyl) on an unsaturated carbon (peak at 111 c.p.s.) atom, and an allenic proton (peak at 365 c.p.s.) in addition to the vinylic proton at C-6. The 21-methyl peak appeared as a symmetrical doublet ($J = 2.5$ c.p.s.) due to the coupling of the 21-methyl protons with the allenic proton, the resonance peak of which appeared as a multiplet. The chloroallene was formed from the tertiary acetylenic carbinol I most possibly by an $\text{S}_{\text{N}}1'$ reaction of the intermediate chlorophosphate or chlorosulfite ester, as shown by Landor and co-workers²³ in analogous cases.



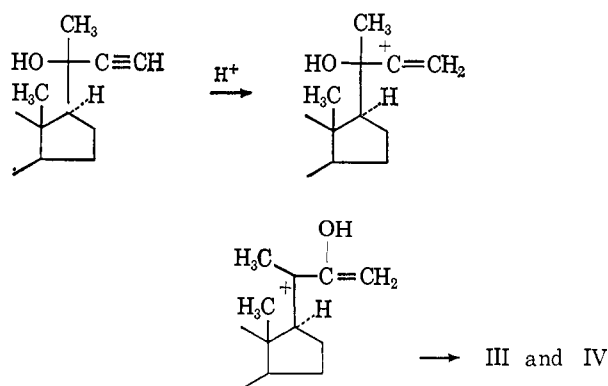
The structure VI and VII are based on their ultraviolet absorption spectra, both of which showed the maximum at 233 $\text{m}\mu$ (ϵ 15,000). Moreover, their n.m.r. spectra showed the presence of a methyl group on an unsaturated carbon atom (21-methyl) and absence of vinylic protons other than the one at C-6. The *cis-trans* stereochemistry was determined, as in the cases of the $\Delta^{17(20)}$ -nitriles XVII and XVIII and the $\Delta^{17(20)}$ -methyl ketones III and IV, by the position of the 21-methyl peak in their n.m.r. spectra and the homoallylic coupling constant of the spin-spin splitting of the 21-methyl group with the 16-methylene group. The 21-methyl peak of the higher melting enyne (145–146°) appeared as a triplet ($J = 1.6$ c.p.s.) centered at 113

(23) Y. R. Bhatia, P. D. Landor, and S. R. Landor, *J. Chem. Soc.*, 24 (1959); S. R. Landor and R. Taylor-Smith, *Proc. Chem. Soc.*, 154 (1959).

(17) E. A. Braude and C. J. Timmons, *J. Chem. Soc.*, 3766 (1955).
 (18) W. A. Forbes and W. A. Mueller, *Can. J. Chem.*, 33, 1340 (1956).
 (19) R. B. Turner and D. M. Voitle, *J. Am. Chem. Soc.*, 73, 1403 (1951).
 (20) R. I. Erskine and E. S. Waight, *J. Chem. Soc.*, 3425 (1960).
 (21) K. Nakanishi in "Infrared Absorption Spectroscopy," Holden-Day, Inc., San Francisco, Calif., 1962.
 (22) A. E. Gillam and E. S. Stern in "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," 2nd Ed., Edward Arnold, London 1960, pp. 101, 102.

c.p.s. and that of the lower melting enyne (137–139°) appeared slightly broadened at 105 c.p.s. The higher melting enyne may, therefore, be assigned the structure VI, and the lower melting enyne, the structure VII. These structures were also confirmed by converting the higher melting enyne VI to the ketone IV, and the lower melting enyne VII to the ketone III by treating them with formic acid for 3–4 min. on a steam bath. It is interesting to note that the conjugated enyne V on similar treatment remained unchanged.

The results of the above acid-catalyzed hydration experiments may explain why the enyne V and not the ketone XV was present in the formic acid reaction mixture of I. These results also suggest that the ketones III and IV, which may be considered as the Rupe rearrangement products of I, might have been formed from I *via* the enynes VII and VI. It remains, however, to be seen whether this dehydration–hydration sequence is the actual pathway for the formation of III and IV from I or whether they were formed, as suggested by Newman²⁴ in an analogous case, by a 1,2 shift of the hydroxyl group after protonation of the triple bond.



Experimental

20 α -Ethynylpregn-5-ene-3 β ,20 β -diol 3-Acetate (I). This compound was prepared by a procedure essentially identical with that described⁷ before. The only modification employed was the use of tetrahydrofuran instead of ether. I was isolated by direct crystallization from methanol in 60% yield instead of by chromatography of the reaction mixture. The yield was increased by 15% when the residue from the mother liquor was chromatographed on alumina as described⁷ before. It was recrystallized from hexane–acetone, m.p. 193–195°; n.m.r. peaks 60.5 (19-methyl), 65 (18-methyl), 124 (acetate methyl), 106 (hydroxyl H), 155 (ethynyl H), and doublet at 327 c.p.s. (6 H).

Reaction of I with Hot Formic Acid. To 5 g. of I was added 25 ml. of 97% formic acid and the mixture was heated with stirring on a steam bath for 3–4 min. when everything went into solution and a deep violet color developed. The solution was then poured over ice and extracted with methylene chloride. The methylene chloride solution was washed with 2 N sodium carbonate solution and water and dried. After evaporating the solvent, a brown residue was obtained which solidified on standing. It showed four spots on thin layer chromatography.

(24) M. S. Newman, *J. Am. Chem. Soc.*, **75**, 4740 (1953).

Reaction of I with Formic Acid. To 5 g. of I were added 50 ml. of 97% formic acid and 25 ml. of methylene chloride. When the solution was left at room temperature with stirring for 24 hr. it turned pink. It was then worked up as described above.

Reaction of I with Sulfuric–Acetic Acid Mixture. To a mixture of 100 ml. of acetic acid and 1 ml. of sulfuric acid was added 5 g. of I; the mixture was heated on a steam bath for 3–5 min. and was worked up as before.

Isolation of Products. The residue obtained from the above reaction of 5 g. of I was dissolved in 10 ml. of benzene and diluted with 10 ml. of hexane. The solution was then put on a column of 200 g. of alumina (Alcoa or Woelm, activity grade III) prepared in hexane and the material was chromatographically separated into various fractions. The fractions were examined by ultraviolet and infrared spectroscopy and thin layer chromatography.

The first fraction (1 g.) which was eluted from the column with a 1:1 mixture of benzene and hexane showed no absorption in the ultraviolet. It had infrared bands at 3.05 (ethynyl C–H), 4.78 (–C \equiv C–), and 5.78 μ (acetate). It was crystallized from methanol (m.p. 141–142°) and identified as II.

Anal. Calcd. for C₂₅H₃₄O₂: C, 81.92; H, 9.35. Found: C, 82.17; H, 9.50.

The second fraction (300 mg.) which was eluted with benzene showed two spots on thin layer chromatography. It was rechromatographed on 30 g. of alumina to give two products, one of which (200 mg.) was identified as II. The other fraction (50 mg.), characterized as V, was crystallized from methanol; m.p. 125–127°; $\lambda_{\text{max}}^{\text{methanol}}$ 223 m μ (ϵ 9000) and 228 m μ (shoulder); infrared bands at 3.05 (ethynyl C–H), 3.2 (=CH₂), 5.45 (overtone of 10.9 μ), 5.8 (acetate), 6.0 (double bond at C₅–C₆), 6.25 (double bond at C₂₀–C₂₁), and 10.9 μ (=CH₂); n.m.r. peaks at 41 (18-methyl), 61.5 (19-methyl), 122 (acetate methyl), 175 (ethynyl H), doublet at 330 having J = 2.5 c.p.s. (one of the H atoms of =CH₂), and complex two-proton absorption at 314–320 c.p.s. (6-H and the other H atom of =CH₂).

Anal. Calcd. for C₂₅H₃₄O₂: C, 81.92; H, 9.35. Found: C, 81.80; H, 9.10.

The third fraction (1.1 g.) which was eluted from the column with 2–5% ether in benzene was identified as III. It was crystallized from methanol: m.p. 176–178°; $\lambda_{\text{max}}^{\text{methanol}}$ 249 m μ (ϵ 3000); infrared bands at 5.78 (acetate), 5.95 (conjugated carbonyl), and 6.1 μ (conjugated C=C).

Anal. Calcd. for C₂₅H₃₆O₃: C, 78.08; H, 9.44. Found: C, 78.32; H, 9.55.

The fourth fraction (650 mg.) which was eluted from the column with 4–10% ethyl acetate in benzene was oily, and showed two spots on thin layer chromatography. It was rechromatographed on 50 g. of the above alumina and resolved into two fractions, one of which (100 mg.) was identified as III. The other fraction (450 mg.), characterized as IV, was crystallized from methanol: m.p. 119–121°; $\lambda_{\text{max}}^{\text{methanol}}$ 253 m μ (ϵ 9000); infrared bands at 5.78 (acetate), 6.0 (conjugated carbonyl), and 6.25 μ (conjugated C=C).

Anal. Calcd. for C₂₅H₃₆O₃: C, 78.08; H, 9.44. Found: C, 77.89; H, 9.57.

Hydrolysis of II to the 3-OH Compound. To 500 mg. of I was added 10 ml. of 10% methanolic potassium

hydroxide, and the mixture was left overnight at room temperature. It was then diluted with water and extracted with ethyl acetate. The residue, obtained after removal of the solvent, was crystallized from aqueous acetone: m.p. 128–130°; n.m.r. peaks 60.5 (19-methyl), doublet centered at 62 having $J = 6.5$ c.p.s. (17a-methyl), 71.5 (17-methyl), 99 (3-OH), 122 (ethynyl H), and doublet at 325 c.p.s. having $J = 4.5$ c.p.s. (6-H).

Anal. Calcd. for $C_{23}H_{32}O$: C, 85.13; H, 9.94. Found: C, 85.32; H, 9.74.

Beckmann Rearrangement of the Oximes of II and IV. Preparation of the Oximes. The oximes were prepared by refluxing a solution of 500 mg. of the ketone (III or IV) and 300 mg. of hydroxylamine hydrochloride in 5 ml. of pyridine and 25 ml. of 95% ethanol for 12 hr. The solution was cooled and the crystallized oxime was filtered off. It was recrystallized from ethanol. The oxime of III melted at 213–215° dec.

Anal. Calcd. for $C_{25}H_{37}NO_3$: C, 75.15; H, 9.33; N, 3.51. Found: C, 75.34; H, 9.33; N, 3.70.

The oxime of IV melted at 216–218° dec.

Anal. Calcd. for $C_{25}H_{37}O_3N$: C, 75.15; H, 9.33; N, 3.51. Found: C, 75.14; H, 9.21; N, 3.51.

Rearrangement of the Oximes. To a cooled solution of 500 mg. of the oxime (of either III or IV) in 10 ml. of dry pyridine was added 2 ml. of phosphorus oxychloride in 5 ml. of pyridine with stirring, and this solution was kept first in an ice bath for 3 hr. and then overnight at room temperature. The next morning, it was poured onto ice containing 2 *N* sulfuric acid, extracted with ethyl acetate, and washed with water. On removal of the solvent, residue was obtained which had infrared bands at 3.05 (NH), 6.05 (amide I band), and 6.60 μ (amide II band). The crude amide was dissolved in 10 ml. of acetic acid and 1 ml. of concentrated hydrochloric acid was added to it. The mixture was shaken for 24 hr., then diluted with water, extracted with ethyl acetate, washed to neutrality with 2 *N* sodium carbonate solution, and dried, and the solvent was removed. The residue was purified by chromatography over alumina. The product obtained on chromatography weighed 350 mg. and was crystallized from aqueous ethanol, m.p. 149–151°. Its infrared spectrum was identical with that of authentic 3 β -hydroxypregn-5-en-20-one acetate.

Dehydration of the Cyanohydrin XVI. Preparation of XVII. To a mixture of 30 ml. of pyridine and 16 ml. of phosphorus oxychloride was added 5 g. of XXVI and the solution was heated on a steam bath for 2 hr. It was then worked up by pouring it onto ice, and extracted with ethyl acetate. The residue, obtained after removal of ethyl acetate, was crystallized from methanol. It weighed 4 g. and was recrystallized from methanol: m.p. 176–177°; $\lambda_{\max}^{\text{methanol}}$ 222 μ (ϵ 14,800); infrared band at 4.5 (conjugated nitrile) and 6.12 μ (conjugated C=C); n.m.r. peaks 56 (18-methyl), 61.5 (19-methyl), triplet centered at 115.5 having $J = 1.6$ c.p.s. (21-methyl), 122.5 (acetate methyl), and 321 c.p.s. having $J = 4$ c.p.s. (6-H).

Anal. Calcd. for $C_{24}H_{33}NO_2$: C, 78.43; H, 9.05; N, 3.81. Found: C, 78.71; H, 9.27; N, 3.59.

Isomerization of the Nitrile XVII. Preparation of the Nitrile XVIII. To a solution of 1 g. of XVII in 25 ml. of dimethyl sulfoxide was added 1 g. of potassium

t-butoxide and then the mixture was stirred overnight in a nitrogen atmosphere. The next morning, 5 ml. of acetic acid was added and the solution was diluted with cold water. The mixture was extracted with ethyl acetate and the residue, obtained after removal of the ethyl acetate, was acetylated at room temperature with 5 ml. of acetic anhydride and 10 ml. of pyridine for 16 hr. After the usual work-up, the residue was purified by chromatography over alumina. The nitrile was eluted from the column with 2% ethyl acetate in benzene. The material obtained upon removal of the solvent weighed 600 mg. It was crystallized from methanol: m.p. 212–214°; $\lambda_{\max}^{\text{methanol}}$ 222 μ (ϵ 14,000); infrared band at 4.5 (conjugated nitrile) and 6.1 μ (conjugated C=C), n.m.r. peaks 56 (18-methyl), 62.5 (19-methyl), 107.5 with half-band width of 2 c.p.s. (21-methyl), 122.5 (acetate methyl), and 322 c.p.s. having $J = 4$ c.p.s. (6-H).

Anal. Calcd. for $C_{24}H_{33}NO_2$: C, 78.43; H, 9.05; N, 3.81. Found: C, 78.57; H, 9.06; N, 3.96.

III and IV from XVIII and XVII. A methylmagnesium bromide solution was prepared from 2.88 g. of magnesium and excess methyl bromide in 25 ml. of dry ether. To this was added 3.67 g. of the nitrile (XVII or XVIII) in 50 ml. of anisole and the mixture was heated at 90° for 48 hr. It was then cooled in an ice bath and decomposed with ice and hydrochloric acid with stirring. The solid iminohydrochloride which separated out was filtered off. The solid was dissolved in a mixture of 20 ml. of methanol and 5 ml. of concentrated hydrochloric acid and heated for 10 min. on a steam bath. It was then left at room temperature for 3 hr., diluted with water, and extracted with ethyl acetate. The residue, obtained after removal of the solvent, was acetylated at room temperature with 5 ml. of acetic anhydride and 10 ml. of pyridine for 16 hr. The residue obtained after the usual work-up was chromatographed over alumina. The fraction eluted with 2–5% ether in benzene weighed 1.7 g. It was crystallized from methanol.

The ketone obtained from XVII had the same melting point, infrared, ultraviolet, and n.m.r. characteristics as IV, and the ketone obtained from XVIII had the same melting point and other characteristics as III.

Reaction of I with Thionyl Chloride. The solution of 2 g. of I in 50 ml. of dimethylformamide was cooled in an ice bath when it turned cloudy. Then 2 ml. of thionyl chloride was added to it with stirring over a period of 5 min. when a clear solution was obtained. It was left cooled in an ice bath for 20 min. and then worked up by pouring over ice and extracted with ethyl acetate. The ethyl acetate solution was washed with 2 *N* sodium carbonate solution, water, and salt solution. The solvent was removed after drying the solution with sodium sulfate. The oily residue was then dissolved in hexane and chromatographed over alumina. Elution was done with a 1:1 mixture of hexane and benzene and finally with benzene. The fractions were examined by their infrared absorption spectrum.

The first fraction (75 mg.) was mostly the chloroallene XIX. It was crystallized from methanol, m.p. 137–139°. It had a strong infrared band at 5.15 (allenic double bond), a very weak band at 3.05 (C=C—H), shoulder at 3.3, and bands at 13.2, 13.5, and 13.7 μ (C—Cl); n.m.r. peaks 42.5 (18-methyl),

62.5 (19-methyl), doublet at 112 having $J = 2.5$ c.p.s. (21-methyl), 122.5 (acetate methyl), doublet at 325 (6-H), and a multiplet at 365 c.p.s. (allenic H).

Anal. Calcd. for $C_{25}H_{35}ClO_2$: C, 74.53; H, 8.69; Cl, 8.82. Found: C, 75.04; H, 8.95; Cl, 8.43.

The second fraction (250 mg.) was a mixture of XIX and V.

The third fraction (750 mg.) was mostly V. It was rechromatographed and crystallized three times from methanol to give 600 mg. of pure V.

The fourth fraction (100 mg.) was crystallized twice from methanol, m.p. 137–139°. It was identified as VII. It had infrared bands at 3.05 and 4.8 μ ; $\lambda_{\max}^{\text{methanol}}$ 233 $m\mu$ (ϵ 15,000); n.m.r. peaks 55 (18-methyl), 62.5 (19-methyl), 105 with half-band width of 2 c.p.s. (21-methyl), 177.5 (ethynyl H), and doublet at 322.5 c.p.s. having $J = 4.5$ c.p.s. (6-H).

Anal. Calcd. for $C_{25}H_{34}O_2$: C, 81.92; H, 9.35. Found: C, 81.78; H, 9.05.

The fifth fraction (125 mg.) was crystallized from methanol: m.p. 145–147°; $\lambda_{\max}^{\text{methanol}}$ 233 $m\mu$ (ϵ 15,000). It was identified as VI. It had infrared bands at 3.05 and 4.8 μ ; n.m.r. peaks at 55 (18-methyl), 62.5 (19-methyl), triplet at 113 having $J = 1.6$ c.p.s. (21-methyl), 182.5 (ethynyl H), and doublet at 322 c.p.s. having $J = 4.5$ c.p.s.

Anal. Calcd. for $C_{25}H_{34}O_2$: C, 81.92; H, 9.35. Found: C, 81.72; H, 9.41.

20-Acetyl-5,20(21)-pregnadien-3 β -ol Acetate (XV).

A catalyst was prepared by warming together 0.25 g. of red mercuric oxide, 0.1 ml. of boron trifluoride etherate, 5 mg. of trichloroacetic acid, and 1 ml. of methanol. A solution of 500 mg. of the vinylacetylene V in 20 ml. of methanol was then added to it and the mixture stirred at room temperature for 3 hr., after which time it was poured into dilute sulfuric acid and the product was isolated by extraction with ethyl acetate. The residue obtained after removal of ethyl acetate was acetylated with 3 ml. of acetic anhydride and 6 ml. of pyridine at room temperature for 16 hr. The product, obtained after working up, was purified by chromatography over alumina. The product (300 mg.) eluted from the column was crystallized from methanol: m.p. 210–211°; $\lambda_{\max}^{\text{methanol}}$ 227 $m\mu$ (ϵ 9500); infrared bands at 5.78 (acetate), 5.98 (conjugated ketone), and 6.15 μ (conjugated C=C).

Anal. Calcd. for $C_{25}H_{36}O_3$: C, 78.08; H, 9.44. Found: C, 77.95; H, 9.26.

Hydration of the Enynes VI and VII. To 1 ml. of 97% formic acid was added 50 mg. of the enyne (VI or VII) and then it was heated on a steam bath for 3 min. when it went into solution and turned pink. It was then worked up by diluting with water and extracting with methylene chloride. After removal of solvent, the crude residue was purified by crystallization. The ketone obtained from VI had the same characteristics as IV, and that from VII was identical with III.

Characterization of Amino Sugars by Mass Spectrometry

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Amino sugar dithioacetals in the form of the N-acetates are suitable derivatives for characterization by mass spectrometry. The classes investigated include derivatives of 2-amino-2-deoxy-, 2,6-diamino-2,6-dideoxy-, 3,6-diamino-3,6-dideoxy-, 3-amino-3,6-dideoxy-, 6-amino-6-deoxyhexoses, and 3-amino-3-deoxy-, 4-amino-4,5-dideoxy-, 5-amino-5-deoxy-, and 4,5-diamino-4,5-dideoxypentoses. The mass spectra are discussed and interpreted in terms of the structures of the acetamido-deoxyaldose diethyl dithioacetals, in terms of the peak shifts in the spectra of the di-n-propyl dithioacetals and the acetamido- d_3 analogs, and in terms of metastable ion peaks.

Introduction

The application of mass spectrometry to carbohydrate chemistry should prove increasingly valuable to the biological field. The limitation of volatility has been overcome by the availability of mass spectrometers with inlet systems that allow direct introduction of the sample into the ion source. The great variety of structure found in the carbohydrate class has also

limited utilization of mass spectrometry in structural carbohydrate chemistry. It has been necessary to systematically study the electron-impact behavior of model compounds, a goal toward which steps have been undertaken during the past 2 years.¹

Initial efforts by Reed and co-workers² were directed to the study of some disaccharides, oligosaccharides, and glycoside derivatives. More detailed studies on the acetates,^{3,4} acetates and methyl ethers,⁵ and isopropylidene derivatives⁶ of pentoses and hex-

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