The Stereochemistry of Dehydrogenation of Δ^4-3-Keto Steroids by Chloranil[1]

J. C. ORR AND J. M. BROUGHTON

John Collins Warren Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University at the Massachusetts General Hospital 02114, and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

Received September 28, 1969

Dehydrogenation of androst-4-ene-3,17-dione-7β-*d* with chloranil gives androsta-4,6-diene-3,17-dione with almost complete retention of the deuterium. Taken with other evidence, this experiment establishes that chloranil abstracts the 7α hydrogen. The mass spectra of steroid 4,6-dien-3-ones show two important peaks corresponding to cleavage of the molecule in ring B. Allylic bromination of 3β-acetoxyandrost-5-en-17-one gives a mixture of the 7α- and 7β-bromo derivatives in approximately equal yield.

The dehydrogenation of Δ^4-3-keto steroids with chloranil to the corresponding 4,6-dien-3-ones was first described by Agnello and Laubach,[2] who proposed the presently accepted mechanism of hydride abstraction from C-7 of the 3,5-dien-3-ol.

Although the dehydrogenation had been shown to involve the loss of the 6β hydrogen atom,[3] the removal of the 7α hydrogen had not been established beyond doubt. The evidence for removal of the 7α hydrogen rested on the observation that, while 7β,17α-dimethyl-17β-hydroxyandrost-4-en-3-one (**1a**) reacts with chloranil in boiling *t*-butyl alcohol to form the 4,6-dien-3-one (**2a**), the 7 epimer (**1b**) reacts much more slowly, if at all, under the same conditions.[4] The presence of the 7-methyl groups, however, may cause disturbance of the ring geometry.

When 3β-hydroxyandrost-5-en-17-one is reduced with tritium gas, some tritium is introduced at the 7 position in the major product, 3β-hydroxy-5α-androstan-17-one. Having established that the tritium at the 6 position was entirely α oriented, Brodie, *et al.*,[3] showed that the C-7 tritium could be removed by dehydrogenation of the derived androst-4-ene-3,17-dione with chloranil. They cited the evidence of Campbell and Babcock[4] and suggested that, by analogy with the *trans* diaxial 1α,2β dehydrogenation of 3-keto steroids to the Δ^1-3 ketones by dichlorodicyanoquinone,[5] the chloranil reaction should remove the 7α axial hydrogen. The *trans* diaxial relation of the two hydrogens removed is, however, of little relevance, since the first step is the preferential[6] loss of the 2β and 6β protons to form the enols. The removal of the 1α hydrogen by dichlorodicyanoquinone could be due to an α-face attack on the Δ^2 enol and need not be due to its axial character. The evidence for removal of the 7α hydrogen during the chloranil reaction was therefore persuasive but not conclusive. Since this reaction was to be used in examining the stereochemistry of deuteron attack at C-7 on the 3α,5α-cycloandrost-6-ene system,[7] it was considered worthwhile to establish the stereochemistry of the chloranil reaction at the 7 position by synthesis and dehydrogenation of a 7-deuterioandrost-4-ene-3,17-dione of known configuration.

Results and Discussion

Treatment of 3β-acetoxyandrost-5-en-17-one (**3a**) with N-bromosuccinimide gave as a crystalline product a mixture of 3β-acetoxy-7α-bromoandrost-5-en-17-one (**3b**) and the 7β-bromo epimer (**3c**) as judged by the nmr spectrum. Recrystallization from ethyl acetate or from carbon tetrachloride gave the pure 7α-bromo isomer (**3b**). Recrystallization of the allylic bromide mixture from alcohols caused accelerated decomposition. The nmr spectrum of the product showed the 6-vinyl hydrogen as a sharp doublet at δ 5.85 ppm (J = 5 Hz) indicative[8] of coupling only to a 7β H, with a dihedral angle (6,7β H) of 40°. The singlet at δ 5.41 ppm (H-6) of the 7β-bromo derivative **3c** was now absent. The H-6–H-7α dihedral angle is *ca.* 80° and would correspond to $J < 1$ Hz. Reduction of the product with lithium aluminum deuteride gave, after crystallization, androst-5-ene-3β,17β-diol-7β,17α-d_2 (**4a**). Combined gas–liquid chromatography–mass spectrometry showed the product to consist of 6% d_1 and 94% d_2 species. The nmr spectrum of the derived diacetate differed from that of androst-5-ene-3β,17β-diol diacetate only in the absence of the H-17α triplet and the appearance of the 6-vinyl proton as a sharp singlet at δ 5.37 ppm ($W_{1/2}$ = 3 Hz), demonstrating that inversion of configuration at C-7 had occurred. Chromium trioxide oxidation of the diol **4a** gave androst-5-ene-3,17-dione-7β-*d*, showing again H-6 as a singlet in the nmr spectrum. Treatment with acid gave the conjugated androst-4-ene-3,17-dione-7β-*d* (**1c**, 96% d_1). During the chromium trioxide oxidation, androst-4-ene-3,6,17-trione containing no excess deuterium was obtained as a minor product. The absence of deuterium at C-7 in the trione, taken together with the observed presence of the vinyl hydrogen at the 6 position in the diol **4a** and in the nonconjugated dione, establishes that the deuterium atom had originally been present at C-7.

In a parallel series of reactions, 17-cycloethylenedioxyandrost-5-en-3β-ol acetate (**3d**) was converted into the bromo derivative **3e** and reduced with lithium aluminum deuteride to 17-cycloethylenedioxyandrost-5-en-3β-ol-*d* (**4b**). When sufficiently purified allylic bromide **3e** was used for the reduction, a by-product,

(1) This is publication No. 1368 of the Cancer Commission of Harvard University. This work was presented in part: Abstracts, 156th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1968, No. ORGN 55.

(2) E. Agnello and G. Laubach, *J. Amer. Chem. Soc.*, **82**, 4293 (1960).

(3) H. J. Brodie, S. Baba, M. Gut, and M. Hayano, *Steroids*, **6**, 659 (1965).

(4) J. A. Campbell and J. C. Babcock, *J. Amer. Chem. Soc.*, **81**, 4069 (1959).

(5) H. J. Ringold, M. Gut, M. Hayano, and A. Turner, *Tetrahedron Lett.*, **18**, 835 (1962).

(6) S. K. Malhotra and H. J. Ringold, *J. Amer. Chem. Soc.*, **86**, 1997 (1964); A. B. Turner and H. J. Ringold, *J. Chem. Soc., C*, 1720 (1967).

(7) J. C. Orr and J. M. Broughton (née Lundgren), manuscript in preparation.

(8) M. Karplus, *J. Amer. Chem. Soc.*, **85**, 2870 (1963).

17-cycloethylenedioxyandrost-6-en-3β-ol-5α-*d*,[9] could not be detected. This by-product may therefore originate from the rearranged 5-bromoandrost-6-ene system. The Δ⁵ ketal **4b** was converted into androst-4-ene-3,17-dione-7β-*d* (**1c**, 100% d_1).

Dehydrogenation of two samples of androst-4-ene-3,17-dione-7β-*d* (**1c**, 96 and 100% d_1), with chloranil gave androsta-4,6-diene-3,17-dione-7-*d* (**2b**, 91 and 95% d_1, respectively), establishing that the dehydrogenation does indeed cause removal of the 7α rather than the 7β hydrogen. The 5% loss of deuterium may be due to 5% androst-4-ene-3,17-dione-7α-*d* in the starting material for the dehydrogenation. This level of contamination would not have been detected in the nmr spectra of the bromo or deuterio compounds. In the chloranil dehydrogenation of androst-4-ene-3,17-dione-7α-*t*, which Brodie *et al.*,[3] used to assign stereochemistry at the 7 position, all but 2% of the starting radioactivity was lost.

The mass spectrum of androsta-4,6-diene-3,17-dione (**2c**, Figure 1) shows two intense fragments at *m/e* 136 and 149. Since in the spectrum of the related 17 alcohol *m/e* 136 remains unchanged but *m/e* 149 has been replaced by a fragment of *m/e* 151, these two peaks may represent cleavage of the molecule at C-1–C-10 and C-7–C-8, *m/e* 136 being the AB end (+2 H) and *m/e* 149 the CD region (−1 H). Introduction of the 7-deuterium atom (**2b**, Figure 2) shifts *m/e* 136 to 137 but leaves *m/e* 149 unchanged.

Allylic bromination of cholesteryl benzoate has been noted[10] to give initially the 7β-bromo compound, which epimerizes to an equilibrium mixture with the 7α-bromo isomer. In an attempt to prepare directly androst-4-ene-3,17-dione-7α-*d*, the product of N-bromo succinimide bromination of androst-5-ene-3β,17β-diol diacetate was immediately evaporated to dryness at room temperature or below and reduced with lithium aluminum deuteride in tetrahydrofuran to give androst-5-ene-3β,17β-diol-7ξ-*d* (7% d_0, 78% d_1, 15% d_2). Oxidation, conjugation, and treatment of the product with chloranil gave androsta-4,6-diene-3,17-dione containing 54% d_1. The bromination product at the time of reduction was thus *ca.* a 1:1 mixture of the 7α- and 7β-bromo isomers. In order to determine whether the initial bromination product had been entirely the 7β-bromo compound which had partly epimerized while refluxing in tetrahydrofuran with lithium aluminum deuteride, 3β-acetoxyandrost-5-en-17-one in an nmr tube was brominated with N-bromosuccinimide and light. After 2 min of heating and irradiation, the nmr peak of the vinyl 6 hydrogen of 3β-acetoxyandrost-5-en-17-one had disappeared completely and had been replaced by the vinyl proton pattern characteristic of a mixture of approximately equal quantities of 7α-bromo- and 7β-bromo-3β-acetoxyandrost-5-en-17-one. This ratio remained unchanged on further irradiation. The presence of a 3β-benzoyloxy group had been claimed to affect the stability of the allylic bromides.[11] Repetition of the bromination using 3β-benzoyloxyandrost-5-en-17-one gave the 7α- and 7β-bromo derivatives in approximately the same ratio.

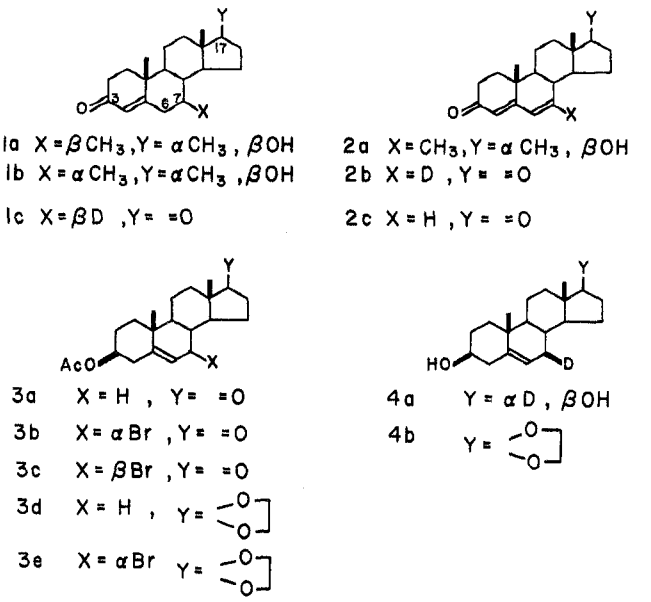


Figure 1.

It is probable that the isolation of 3β-benzolyloxy-7β-bromocholest-5-ene by Schaltegger[12] was due to its solubility characteristics rather than to selective 7β bromination.

Experimental Section

Combined gas–liquid chromatography–mass spectrometry was carried out on an LKB 9000 instrument with helium carrier gas, and OV-1 on Gas-Chrom Q (Applied Science Laboratories, State College, Pa.) at 200° as the stationary phase. Samples in the solid state, adsorbed on stainless steel gauze,[13] were injected into the flash heater at 230°. The molecular separator was maintained at 250° and the ion source at 270°. The mass spectrometer ionizing current was 50 μA, and the ionizing energy was 70 eV during the mass spectral scans. Melting points were determined on a Kofler block and are not corrected. Nmr spectra were measured on a Varian A-60 spectrometer in deuteriochloroform solution unless otherwise noted. Chemical shifts are reported in δ units with tetramethylsilane (δ 0.00 ppm) as internal standard. Evaporations were carried out under vacuum in a rotary evaporator with a bath temperature of *ca.* 45° unless otherwise noted.

3β-Hydroxy-7α-bromoandrost-5-en-17-one Acetate (3b).—3β-Hydroxyandrost-5-en-17-one acetate (10 g, 30.3 mmol) in refluxing carbon tetrachloride (70 ml) dissolved completely. N-Bromosuccinimide (5.35 g, 1.07 equiv) in carbon tetrachloride (30 ml) was added and the mixture was refluxed over a 150-W lamp for 8 min. The yellow solution was filtered to remove the crystalline succinimide and evaporated almost to dryness under reduced pressure without heating to give faintly yellow crystals. Three recrystallizations from carbon tetrachloride–ethyl acetate gave the 7α-allylic bromide (**3b**, 2.7 g, 6.6 mmol, 22% yield): mp 150–153° (lit.[12] mp 155°); nmr δ 5.85 (d, J = 5.0 Hz, H-6), 4.78 (d, J = 5.0 Hz, H-7β), 2.03 (acetate), 1.07 (C-19 H_3), and 0.90 ppm (C-18 H_3).

Androst-5-ene-3β,17β-diol-7β,17α-d_2 (4a).—3β-Hydroxy-7α-bromoandrost-5-en-17-one acetate (**3b**, 1.5 g) in dry tetrahydrofuran (120 ml) was refluxed for 5 hr with lithium aluminum deuteride (1.0 g, Alfa Inorganics, 99+% *d*). Thin layer chromatography showed the reduction to be complete. Water and ether were added, and the organic layer was separated. The aqueous layer was reextracted twice with ether, and the combined organic layers were washed with water and evaporated to a crystalline mass, which upon recrystallization gave androst-5-ene-3β,17β-

(9) C. Djerassi, G. von Mutzenbecker, J. Fajkos, D. H. Williams, and H. Budzikiewicz, *J. Amer. Chem. Soc.*, **87**, 817 (1965).

(10) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 159.

(11) H. Schaltegger, *Helv. Chim. Acta*, **33**, 1316 (1950).

(12) R. Antonucci, S. Bernstein, D. Giancola, and K. J. Sax, *J. Org. Chem.*, **16**, 1126 (1951).

(13) (a) E. Menini and J. K. Norymberski, *Biochem. J.*, **95**, 1 (1965); (b) E. Menini, J. C. Orr, R. Gibb, and L. L. Engel in "Gas Chromatography of Steroid Hormones," R. Scholler, Ed., Gordon and Breach, New York, N. Y., 1968, pp 41–64.

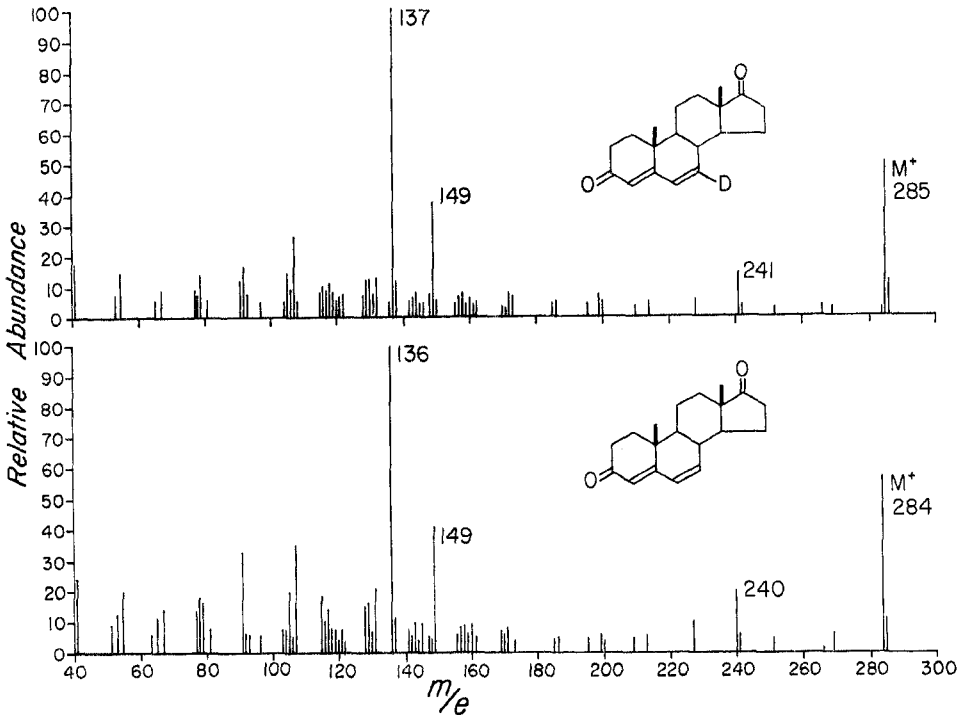


Figure 2.

diol-7β,17α-d_2 (**4a**, 800 mg): mp 178–180.5°, undepressed on mixing with an authentic sample of the unlabeled diol of mp 178–180°; mass spectrum *m/e* 292 (M^+). The nmr spectrum of the diol **4a** in chloroform was unsatisfactory owing to low solubility.

The derived diacetate, mp 159–162°, was undepressed on admixture with an authentic sample of androst-5-ene-3β,17β-diol diacetate, mp 159–162°. The nmr spectrum of androst-5-ene-3β,17β-diol-7β,17α-d_2 diacetate showed the absence of the H-17α triplet and showed a singlet at δ 5.37 ppm ($W_{1/2}$ = 3 Hz, H-6). The mass spectrum showed as the heaviest ion *m/e* 316 (M − 60); the fragmentation pattern corresponds closely to that expected on the basis of the nondeuterated diacetate.

Chromium Trioxide Oxidation of Androst-5-ene-3β,17β-diol-7β,17α-d_2 (4a).—The diol **4a** (100 mg, 0.35 mmol) in acetone was stirred with chromium trioxide (0.195 ml of 8 *N*, 1.4 equiv) in 1 *N* sulfuric acid for 5 min.[14] The brown chromate remained in excess. The solution was extracted with ethyl acetate–water, and the ethyl acetate layer was washed and evaporated to give a crystalline solid. The nmr spectrum showed this to be the nonconjugated androst-5-ene-3,17-dione-7β-*d;* the angular methyl groups (δ 1.21 and 0.88 ppm) and H-6 (δ 5.35 ppm) all appeared as singlets, giving no indication of contamination with the Δ^6 isomer.

Treatment of the nonconjugated dione with methanol (5 ml) containing concentrated hydrochloric acid (1 drop) at room temperature overnight and two recrystallizations from aqueous ethanol gave androst-4-ene-3,17-dione (**1c**): yield 12 mg; mp 172–174°, undepressed on mixing with authentic nonlabeled androst-4-ene-3,17-dione of mp 172–174°. Combined gas–liquid chromatography–mass spectrometry showed the product to be homogenous and to contain 96% d_1.[15] The abundant fragment at *m/e* 124 of ring A, C-6, C-19, and 2 H[16] was not shifted to *m/e* 125 and hence did not contain the deuterium. Glpc of the mother liquors revealed the presence of androst-4-ene-3,6,17-trione [*m/e* 300 (M^+)] as a minor component. The mass spectrum of his compound was identical with that of an authentic sample; it contained no excess deuterium.

7α-Bromo-17-cycloethylenedioxyandrost-5-en-3β-ol Acetate (3e).—17-Cycloethylenedioxyandrost-5-en-3β-ol acetate (17.7 g, 47.3 mmol) in carbon tetrachloride was heated on a steam bath. N-Bromosuccinimide (10.1 g, 1.1 equiv) was added and the mixture was refluxed over a 150-W lamp for 10 min. The solution was filtered and evaporated on a rotary evaporator in a water bath at *ca.* 25° under vacuum until crystals began to form. Ethyl acetate was added and the suspension was left in an ice bath.

Two recrystallizations of the crystalline product, by dissolving carbon tetrachloride, adding ethyl acetate, and rotary evaporation at room temperature or below, gave the allylic bromide **3e** (5.21 g): mp 164–165° (lit.[9] mp 148–151°); nmr δ 0.86 and 1.04 (sharp singlets with no evidence of impurity, angular methyl groups), 3.87 (ketal CH_2), 4.63 (H-7β), and 5.75 ppm (H-6).

17-Cycloethylenedioxyandrost-5-en-3β-ol-7β-*d* (4b).—The allylic bromide **3e** (2.73 g, 6.02 mmol) in tetrahydrofuran (200 ml, freshly distilled from lithium aluminum hydride) was refluxed overnight with $LiAl_4D$ (24 mmol). The solution was diluted with water, and *ca.* 150 ml of tetrahydrofuran were evaporated. The solution was further diluted with water and extracted with ether; the ether solution was washed and evaporated to give colorless crystals of **4b**, recrystallized from methanol, mp 165–168° (1.38 g, 69% yield). In the absence of an authentic nonlabeled sample, the mass spectrum alone does not allow calculation of the per cent deuterium; the peaks in the region of the molecular ion at *m/e* (rel intensity) 232 (8), 233 (100), and 234 (23) indicate that the material is substantially monodeuterated. The substance was homogenous both by glpc and tlc. The nmr spectrum again showed no peak corresponding to the angular methyl groups of the Δ^6 isomer:[9] δ 5.34 (s, H-6), 3.88 (ketal), and 1.01 and 0.86 ppm (angular methyl).

Androsta-4,6-diene-3,17-dione-7-*d* (2b).—Androst-4-ene-3,17-dione-7β-*d* (10 mg, 100% d_1) in benzene (10 ml) with chloranil (50 mg) was refluxed for 2 days. The benzene solution was washed with aqueous KOH and then with water until neutral. A portion of the benzene solution was applied directly to stainless steel gauze and injected into the combined glpc–mass spectrometer. The major glpc peak (85% of the total steroid peak area) corresponded in retention time and mass spectrum to androsta-4,6-diene-3,17-dione-7-*d* (**2d**, Figure 2). Three successive mass spectral scans taken over the peak showed 96.1, 95.2, and 94.2% d_1 (average 95% d_1).[15]

Similar dehydrogenation of the androst-4-ene-3,17-dione-7β-*d* (96% d_1) gave the diene dione **2b**, containing, on successive scans over the peak, 92.1, 91.6, 91.5, 91.5, 89.9, and 88.6% d_1 (average 91% d_1).

N-Bromosuccinimide Bromination of 3β-Benzoyloxyandrost-5-

(14) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(15) As with all steroids so far examined by glpc–mass spectrometry on OV1, the deuterated steroid has a slightly shorter retention time than does the unlabeled molecule. This has been observed with greater clarity in the fatty acid series, where the predeuterated acid methyl esters have been separated completely from the unlabeled material: J. A. McCloskey, A. M. Lawson, and F. A. J. M. Leemans, *Chem. Commun.*, 285 (1967).

(16) R. H. Shapiro and C. Djerassi, *J. Amer. Chem. Soc.*, **86**, 2825 (1964).

en-17-one and of the Corresponding Acetate (3a).—The benzoate (0.10 mmol) in deuteriochloroform containing tetramethylsilane in an nmr tube was refluxed and irradiated after addition of N-bromosuccinimide (0.11 mmol). After 2 min, the doublet at 5.46 ppm (H-6) (7,7 H_2) had diminished while a doublet at δ 5.95 ppm (J = 5.1 Hz), H-6 (7α Br) and a singlet at δ 5.72 ppm (H-6) (7β Br), ratio 1.3:1, had appeared.

A similar bromination of 3β-acetoxyandrost-5-en-17-one (**3a**) in CCl_4 gave a ratio of 7α Br (**3b**) to 7β Br (**3c**) product of 1.2:1.

Registry No.—Chloranil, 118-75-2; **1c**, 23668-15-7; **2b**, 23668-16-8; **2c**, 633-34-1; **3b**, 23668-18-0; **3e**, 748-37-8; **4a**, 23668-20-4; **4a** diacetate, 23668-21-5; **4b**, 23688-22-6.

Acknowledgments.—This work was supported by U. S. Public Health Service (Grants CA02421 and CA01939) and a grant from the American Cancer Society (P95). The LKB 9000 combined glpc–mass spectrometer was purchased through a special grant from the American Cancer Society (Massachusetts Division). The authors are deeply indebted to Dr. L. L. Engel for encouragement and helpful discussion of this work.

The Addition of Coordinated Glycine to Acetaldehyde. Mechanism[1]

D. C. BERNDT

Department of Chemistry, Western Michigan University, Kalamazoo, Michigan 49001

Received October 3, 1969

The kinetics of the reaction of acetaldehyde with glycinatobis(ethylenediamine)cobalt(III) chloride in the presence of a tertiary amine in water solution to produce the threoninato complex ion has been studied. The rate of reaction is first order in aldehyde and one-half order in each the complex ion and the amine base. These results are consistent with a mechanism involving abstraction by base of an α proton of the glycine moiety followed by reaction of the resulting enolatelike ion with aldehyde.

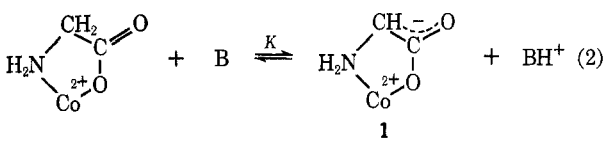
Aldehydes react with glycine coordinated with certain metal ions in the presence of base to produce hydroxy amino acids,[2-6] *e.g.*, eq 1, where en is ethylenediamine and gly and thr are glycine and threonine (allo and

$$(\text{Coen}_2\text{gly})^{2+} + \text{CH}_3\text{CHO} \xrightarrow[\text{H}_2\text{O}]{\text{base}} (\text{Coen}_2\text{thr})^{2+} \qquad (1)$$

threo) anions, respectively, coordinated with cobalt(III). If optically active glycinatobis(ethylenediamine)cobalt(III) ion is treated with acetaldehyde, an asymmetric synthesis of threonine and allothreonine can be effected.[6] The base most commonly used is sodium carbonate. The amino acids are obtained upon cleavage of the ligands from the metal ion.

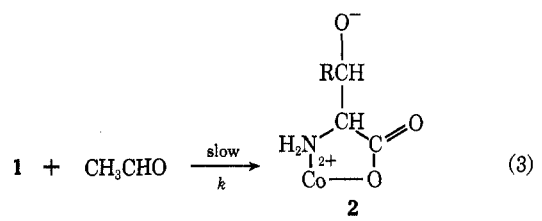
This paper reports the results of a kinetic study of reaction 1 with a tertiary amine, 1,4-diazabicyclo[2.2.2]-octane (dabco), serving as the base and glycinatobis-(ethylenediamine)cobalt(III) chloride and acetaldehyde serving as the reactants. Essentially complete conversion of glycine into the threonines occurs with this base. The results of this study are consistent with the mechanism of eq 2–4 where B is the base and the ethylenediamine ligands are omitted for clarity.

Reaction conditions (Table I) were chosen so that the reaction was pseudo first order in acetaldehyde and such that the competing aldol condensation[7] can be neglected. The spectrophotometric method developed for the determination of aldehyde concentration as a



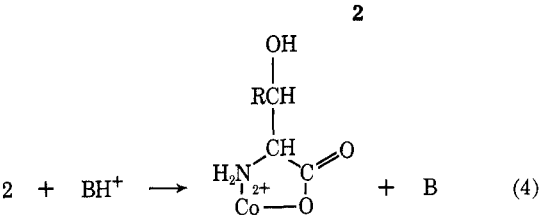
(2)

1



(3)

2



(4)

function of time is described in the Experimental Section. Equation 5 describes the observed rate law at essentially constant ionic strength and chloride ion concentration for the reaction conditions investigated (Table I).

$$\frac{-\text{d}[\text{CH}_3\text{CHO}]}{\text{d}t} = k_1[\text{dabco}]^{1/2}[(\text{Coen}_2\text{gly})\text{Cl}_2]^{1/2}[\text{CH}_3\text{CHO}] \qquad (5)$$

This rate law is related to the above mechanism as shown in eq 6–9; K_B is the basic dissociation constant of

$$\frac{-\text{d}[\text{CH}_3\text{CHO}]}{\text{d}t} = k[\text{CH}_3\text{CHO}][1] \qquad (6)$$

$$= k[\text{CH}_3\text{CHO}]K[(\text{Coen}_2\text{gly})^{2+}][\text{B}]/[\text{BH}^+] \qquad (7)$$

$$[\text{BH}^+] = [^-\text{OH}] + [1] \qquad (8)$$

$$\text{or } [\text{BH}^+]^2 = [\text{B}]\{K_B + K[(\text{Coen}_2\text{gly})^{2+}]\} \qquad (9)$$

dabco. The contribution to $[BH^+]$ from the formation of $CH_3CH(OH)O^-$ can be neglected since the known values at 25° of the hydration constant[7] of acetaldehyde

(1) The support of this work by the U. S. Public Health Service, National Institute of Arthritis and Metabolic Diseases Grant AM 12262, is gratefully acknowledged.

(2) M. Sato, K. Okawa, and S. Akabori, *Bull. Chem. Soc. Jap.*, **30**, 937 (1957).

(3) S. Akabori, T. T. Otani, R. Marshall, M. Winitz, and J. P. Greenstein, *Arch. Biochem. Biophys.*, **83**, 1 (1959).

(4) T. T. Otani and M. Winitz, *ibid.*, **102**, 464 (1963).

(5) Y. Ikutani, T. Okuda, M. Sato, and S. Akabori, *Bull. Chem. Soc. Jap.*, **32**, 203 (1959).

(6) M. Murakami and K. Takahashi, *ibid.*, **32**, 308 (1959).

(7) R. P. Bell and P. T. McTigue, *J. Chem. Soc.*, 2893 (1960).