n.m.r., and mass spectra, t.l.c. behavior, and color reaction essentially identical with those of the material obtained by degradation of neblinine (IIe).

Lithium Aluminum Hydride Reduction of O-Methyl-N-depropionylaspidoalbine.[17]—O-Methyl-N-depropionylaspidoalbine (60 mg., m.p. 147–148°) was reduced 1.5 hr. with lithium aluminum hydride (50 mg.) in refluxing ether (10 ml.); the product (65 mg.) was purified on a 22-mm. dry-packed partition column containing 30 ml. of the lower phase of the system hexane–ethylene dichloride–methanol–water (40:20:8:1) on 45 g. of Celite 545. Product A (19-epi-VIa, 6 mg., amorphous) appeared at R_f 0.50; product B (VIa, 52 mg.) appeared at R_f 0.40, and was crystallized from acetone–isopropyl ether to give 24 mg., m.p. 154–155°; mass spec. *m/e* 388 (M^+), 370 ($M^+ - H_2O$), 360 ($M^+ - CH_2{=}CH_2$), 140 (ion s).

Product isomer A showed mass spec. *m/e* 388 (M^+), 387 ($M^+ - 1$) while the above-mentioned peaks occurred at much lower intensity.[17]

[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, SHREWSBURY, MASS.]

Steroids Containing Ring A Aromatic. VIII. Mechanism of Dienone–Phenol Rearrangement[1]

BY E. CASPI,[2a] P. K. GROVER,[2b] AND Y. SHIMIZU[2c]

RECEIVED JANUARY 27, 1964

Conclusive proof is provided for the mechanism of dienone–phenol rearrangements of steroids. Formation of I proceeds through the migration of the 19-methyl from C-10 to C-1. The route to phenols II is initiated by the rupture of the C-9(10) bond, followed by formation of intermediate III. Subsequently, migration and attachment of C-9 to the C-4 of the dienone occurs.

The versatility[3] and biosynthetic implications[4] are probably the reasons for the continuous interest in the chemistry of the dienone–phenol rearrangements. By varying reaction conditions, acid catalysts, or substituents on the substrate, either phenols of type I or II are obtained as predominant products[5] from the rearrangement of steroidal 1,4-dien-3-ones. It is usually assumed that *meta* derivatives I are formed by a shift of the C-10 methyl to C-1 and *para* derivatives II through intermediate III. Though a large body of indirect evidence supporting these assumptions was accumulated in recent years, unequivocal proof was lacking. It was therefore considered relevant to establish conclusively the pathways of the rearrangement. The approach we chose was to rearrange 4-C^{14}-labeled steroidal dienones, degrade the appropriate ring A phenols, and locate the tracer.

In considering possible pathways leading from dienones to 3-hydroxy-1-methyl-1,3,5(10)-trienes, an alternative route[6] can be visualized in addition to a C-10 to C-1 shift of the angular methyl. If the 19-methyl migrates to C-5 after the initial protonation of the carbonyl, formation of intermediate IV could occur. Subsequent migration of the C-6 bond rather than the C-9 would yield phenol I, which would contain the tracer at C-2. Should the C-10 to C-1 shift of the methyl occur, the distribution of ring A atoms in both the dienone and the phenol would remain the same. Obviously by utilizing a 4-C^{14}-dienone, the problem lends itself to a facile solution. All that is required is to locate the tracer in the *m*-phenol I. Since the 3-hydroxy-1-methyl-1,3,5(10)-trienes can be obtained from both the 1,4-dien-3-one and 1,4,6-trien-3-one, it was deemed necessary to investigate the mechanism in both cases.

For the present studies 4-C^{14}-testosterone acetate (Va) was dehydrogenated[7,8] to yield the 1,4-dienone Vb. Saponification of Vb gave Vc which was oxidized to Vd. The dienedione Vd was treated with aqueous acetic acid–hydrochloric acid[9] giving a mixture of *p*-(1,4) and *m*-(1,3) phenols from which Ia was obtained after saponification. Alternatively, 4-C^{14}-testosterone acetate (Va) was converted in two steps[8,10] to the 1,4,6-trien-3-one Ve which was saponified and oxidized to Vf. The trienedione Vf was rearranged with acetic anhydride–*p*-toluenesulfonic acid.[11] The obtained methylphenol acetate Ib was reduced catalytically, then saponified to provide Ia. The phenols Ia, obtained through the two routes, were converted *via* Birch reduction of their methyl ethers to 17β-hydroxy-1α-methylestr-4-en-3-one[12] (VIa). Ozonolysis of the acetates VIb provided formic acid[13,14] derived from C-4. Subsequently, the formic acid was oxidized to carbon dioxide, which was isolated as barium carbonate. From the nonvolatile ozonization residue the lactol[15] VII was recovered. The distribution of radioactivity in the products is summarized in Table I.

It is apparent that in both cases the radioactivity was located at C-4 of 17β-acetoxy-1α-methylestr-4-en-3-one(VI), independent of whether it was derived from dienone Vd or trienone Vf. Thus, it can be concluded with certainty that in both cases the rear-

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(3) For leading reference, see P. J. Kropp, *J. Am. Chem. Soc.*, **85**, 3280 (1963); B. R. Davis and T. G. Halsall, *J. Chem. Soc.*, 1833 (1962).

(4) D. H. R. Barton and T. Cohen in "Festschrift, Professor A. Stoll," Birkhauser Publishers, Basel, 1957, p. 124; W. Weis, Ch. Gilwarg, E. S. Mingioli, and B. D. Davis, *Science*, **119**, 774 (1954).

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(10) H. J. Ringold and A. Turner, *Chem. Ind.* (London), 211 (1962).

(11) C. Djerassi, G. Rosenkranz, J. Romo, J. Pataki, and S. Kaufmann, *J. Am. Chem. Soc.*, **72**, 4540 (1950).

(12) H. J. Ringold, G. Rosenkranz, and F. Sondheimer, *ibid.*, **78**, 2477 (1956).

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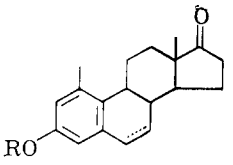
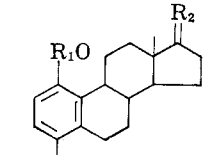
(14) E. Caspi, "Biosynthesis of Lipids," G. Popjak, Ed., Proceedings of Fifth International Congress of Biochemistry, Moscow, 1961, Pergamon Press, London, and P. W. N. Polish Scientific Publishers, Warsaw, 1963, Vol. VII, p. 347.

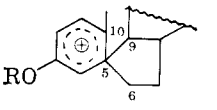
(15) E. Caspi, W. Schmid, and B. T. Khan, *Tetrahedron*, **18**, 767 (1962).



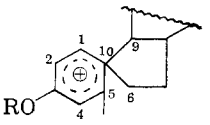
Ia, R = H
b, R = CH_3·CO; Δ^6
c, R = CH_3



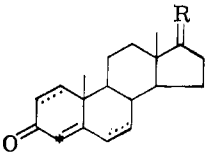
IIa, R_1 = CH_3·CO; R_2 = O
b, R_1 = H; R_2 = OH(β),H



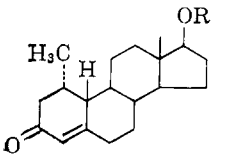
III



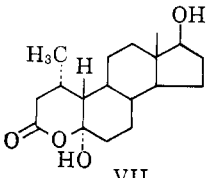
IV



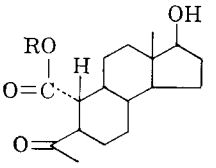
Va, R = O·CO·CH_3(β),H
b, Δ^1; R = O·CO·CH_3(β),H
c, Δ^1; R = OH(β),H
d, Δ^1; R = O
e, $\Delta^{1,6}$; R = O·CO·CH_3(β),H
f, $\Delta^{1,6}$; R = O



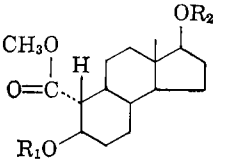
VIa, R = H
b, R = O·CO·CH_3



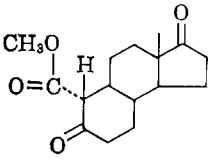
VII
+
$HCOOH \longrightarrow CO_2 \longrightarrow BaCO_3$
(4)



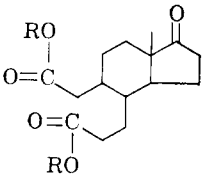
VIIIa, R = H
b, R = CH_3



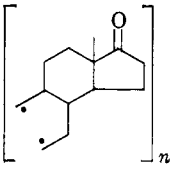
IXa, R_1 = CH_3CO; R_2 = CF_3CO
b, R_1 = R_2 = H



X



XIa, R = H
b, R = CH_3



XII

TABLE I

DISTRIBUTION OF C^{14} IN PRODUCTS DERIVED FROM 3-HYDROXY-1-METHYLESTRA-1,3,5(10)-TRIENE-17-ONE (Ia)

	Specific activity, counts/min./mmole × 10^3	
	Compound rearranged	
Compound	Vd (dienone)	Vf (trienone)
VI	42	50
VII	None	None
$BaCO_3$	39	48.5

rangement leading to 3-hydroxy-1-methyl-1,3,5(10)-trienes proceeds by an analogous route, namely, scission of the C-10(19) bond and migration of the methyl to C-1. This excludes the alternative mechanism involving intermediate IV.

Having completed the investigation of the *m*-phenols I, we turned our attention to the mechanism involving formation of 1-hydroxy-4-methyl-1,3,5(10)-trienes II. The approach selected was similar to that used above, namely, to compare the distribution of ring A carbon atoms in the dienone and the derived *p*-phenol II. Should intermediate III be involved in the rearrangement, the C-4 of the dienone Vd would be located at C-10 of the phenol II. Consequently the 4-C^{14}-dienone Vd was rearranged with zinc chloride–acetic anhydride,[16] and the product Ia was reduced with lithium aluminum hydride to yield IIb. The phenol was oxidized with alkaline hydrogen peroxide to yield among others the 2,3-bisnor acid[17,18] VIIIa. Its ester VIIIb was submitted to Baeyer–Villiger oxidation with trifluoroperacetic acid to provide IXa. Saponification and treatment with diazomethane led to diol IXb, which was oxidized to dione[18,19] X. Cleavage of the β-keto ester X with methanolic potassium hydroxide gave the dicarboxylic acid[19] XIa, which was converted to the diester XIb. Electrolysis[13,14,20] of XIa yielded carbon dioxide, isolated as barium carbonate (78%), derived from both C-1 and C-5.

(16) A. S. Dreiding and A. Voltman, *J. Am. Chem. Soc.*, **76**, 537 (1954).
(17) E. Caspi, P. K. Grover, and D. M. Piatak, *Chem. Ind.* (London), 1495 (1963). Compound II in the reference should read λ_{max}^{MeOH} 215 mμ.
(18) E. Caspi, P. K. Grover, D. M. Piatak, and Y. Shimizu, *J. Chem. Soc.*, in press.
(19) F. Sondheimer, R. Mechoulam, and M. Sprecher, *Tetrahedron Letters*, No. **22**, 38 (1960).
(20) J. W. Cornforth, I. Youhotsky, and G. Popjak, *Biochem. J.*, **65**, 94 (1957).

On processing the residue from the electrolytic decarboxylation, a neutral fraction was obtained which can be presented schematically as XII.

The products VIIIb, IXa, IXb, X, XIa, the barium carbonate, and the residue XII were assayed for their C^{14} content. The results are summarized in Table II. It is apparent that carbons 1, 2, 3, 4, and

TABLE II

SPECIFIC ACTIVITY OF DEGRADATION PRODUCTS OF 1,17β-DIHYDROXY-4-METHYLESTRA-1,3,5(10)-TRIENE (IIb) SYNTHESIZED FROM 4-C^{14}-ANDROSTA-1,4-DIENE-3,17-DIONE (Vf)

Compound	Specific activity,[a] counts/min./mmole × 10^3	Compound	Specific activity,[a] counts/min./mmole × 10^3
Ib	125	X	125
VIIIb	115	XI	129
IXa	125	$BaCO_3$ (C-1 + C-5)	1.2
IXb	119	XII	98

[a] All counts are corrected for the same dilution and thickness on the planchets.

5 and the "19"-methyl are devoid of radioactivity and that the tracer is located at C-10. This is fully corroborated by finding the C^{14} in XII. The fact that XII contains only 80% of the expected radioactivity is very likely the result of difficulties in purifying and drying the viscous sirup. The finding of about 1% of carbon-14 in the carbon dioxide derived from C-1 and C-5 can be rationalized by assuming a partial anodic oxidation of XII (or its analog) followed by decarboxylation. The evidence presented constitutes conclusive proof that C-4 of androsta-1,4-diene-3,17-dione became C-10 in the 1-hydroxy-4-methyl-1,3,5(10)-triene II.

It has been suggested that the more substituted carbon in the spiro intermediate III migrates.[21] The formation of 1-acetoxy-4,6α-dimethylestra-1,3,5(10)-trien-17-one as the sole product of reaction of 6α-methylandrosta-1,4-diene-3,17-dione with acetic anhydride and *p*-toluenesulfonic acid was recently reported.[22] This observation raises the question whether the degree of substitution is the sole factor controlling the migrating aptitude of a carbon. The fact that 4-hydroxy-1-methylphenols have not yet been found among products of rearrangement of C-6 substituted steroidal 1,4-dien-3-ones is rather puzzling. It is apparent that presently available evidence does not allow a distinction of the more subtle aspects of the mechanism. However, the assumption that the rearrangement is initiated by an attack on the carbonyl, followed by formation of a cation, can be suggested with considerable certainty. Which of the two bonds in the *p*-position, the C-9(10) or C-10(19), will break seems to depend on the nature of the dienone and the conditions of rearrangement. Since the gross aspects of the dienone–phenol rearrangement have now been solved, attention can be focused on elucidation of the factors influencing bond cleavage and bond migration.

Experimental[23]

Preparation of 4-C^{14}-3-Hydroxy-1-methylestra-1,3,5(10)-trien-17-one (Ia) from 4-C^{14}-Androsta-1,4-diene-3,17-dione (Vd).—A mixture of 4-C^{14}-testosterone acetate (400 mg.), dry dioxane (25 ml.), and 2,3-dichloro-5,6-dicyanobenzoquinone (400 mg.) was refluxed for 22 hr. with exclusion of moisture.[8] After the conventional work-up, 400 mg. of a colorless sirup was obtained. The sirup was dissolved in methanol (10 ml.), the air was displaced with nitrogen, and 2 *N* sodium hydroxide (5 ml.) was added. The mixture was then stored for 16 hr. at room temperature. The product Vc was recovered with ether–methylene chloride (3:1) from the water-diluted and acidified reaction mixture. The extract was washed, dried, and concentrated to a residue. The residue was dissolved in pyridine (5 ml.) and added to a suspension of chromium trioxide (400 mg.) in pyridine (5 ml.). After 3 hr. at ambient temperature, ethyl acetate was added, and the solids were removed by filtration on Celite. The filtrate was washed, dried, and concentrated to yield androsta-1,4-diene-3,17-dione (Vd). A solution of dione Vd in acetic acid (6 ml.), water (1 ml.), and concentrated hydrochloric acid (2 ml.) was refluxed for 6 hr. Dilution with water provided a solid which was collected by filtration. The solid was suspended in 2 *N* sodium hydroxide and heated on a steam bath for 1 hr. The insoluble portion was separated by filtration, and the leaching was repeated. From the acidified first filtrate, the phenol Ia was recovered with methylene chloride–ether (1:3). After washing with water, the volume of the extract was reduced *in vacuo* to yield Ia (75 mg.), identical with an authentic sample. From the acidified filtrate of the second "leaching" a sirup was recovered and combined with the above mother liquor. Chromatography on a column of silica gel and percolation with benzene yielded an additional amount of Ia (67 mg.).

Preparation of 4-C^{14}-3-Hydroxy-1-methylestra-1,3,5(10)-trien-17-one (Ia) from 4-C^{14}-Androsta-1,4,6-triene-3,17-dione (Vf).—A sample of 4-C^{14}-testosterone acetate (500 mg.) was converted to the 1,4,6-triene acetate Ve exactly as previously described for testosterone,[8] and the radioactive sample was saponified with aqueous methanolic sodium hydroxide. The infrared spectrum of the free alcohol (400 mg.), m.p. 156–158° (lit.[8] 156–158°), was identical with that of an authentic sample. The alcohol was oxidized with the chromium trioxide–pyridine reagent as described above to yield Vf (350 mg.), m.p. 160–165° (lit.[8] 164–165°). The infrared spectrum of the radioactive trienedione Vf was indistinguishable from that of an authentic sample.[8] Recrystallized (ethyl acetate) Vf (300 mg.) and *p*-toluenesulfonic acid (80 mg.) was dissolved in acetic anhydride (12 ml.), and the solution was heated for 5 hr. on a steam bath. The cooled solution was diluted with water (7 ml.), and after 1 hr. at room temperature was poured on a mixture of ice and a slight excess of a saturated sodium hydrogen carbonate solution. Subsequently, solid Ib was collected by filtration and dried (270 mg.), m.p. 145–150° (lit.[11] 152–153°). The phenol Ib was hydrogenated in ethyl acetate (80 ml.) containing 10% Pd-on-charcoal (200 mg.). Within 90 min., 24 ml. of hydrogen was absorbed, and the reaction was terminated. The usual work-up provided a sirup (300 mg.) which gave 230 mg. of crystals (from methanol), m.p. 155–158°. The obtained solid was dissolved in methanol (15 ml.), and a solution of a sodium methoxide in methanol (2 ml.) was added (1.14 g. of sodium in 10 ml. of methanol). After 10 min. at ambient temperature, acetic acid (8 ml.) and water (10 ml.) were added, and the volume of the mixture was reduced *in vacuo*. On concentration, the phenol Ia crystallized (160 mg.), m.p. 248–252° (lit.[11] 250–252°). Extraction of the mother liquors gave an additional amount of less pure Ia (60 mg.). The infrared spectrum of phenol Ia was indistinguishable from an authentic sample.[11]

17β-Acetoxy-1α-methylestr-4-en-3-one(VIb). (a). From Ia Prepared *via* Dienone Vd.—To a boiling solution of Ia (140 mg.) in methanol (25 ml.) and 10% sodium hydroxide (6 ml.), dimethyl sulfate (1 ml.) was added in several portions. The mixture was refluxed for 30 min.; then 40% sodium hydroxide (2 ml.) and additional dimethyl sulfate (1 ml.) were added. After refluxing was continued for 60 min., the reaction was cooled, water was added, and the steroids were recovered in the conventional manner (120 mg.). Crystallization from acetone–hexane gave

(21) R. B. Woodward in "Perspectives in Organic Chemistry," A. Todd, Ed., Interscience Publishers, Inc., New York, N. Y., 1956, p. 178.

(22) D. Burn, V. Petrow, and G. Weston, *J. Chem. Soc.*, 29 (1962).

(23) Infrared spectra were taken on potassium bromide disks. Ultraviolet spectra (for methanol solutions) were taken on a Cary Model 11 MS or 14 spectrophotometer. Melting points were determined on a micro hot stage and are corrected. Analyses by I. Beetz, Kronach, W. Germany. The samples were counted exactly as previously described: E. Caspi, *et al.*, *J. Biol. Chem.*, **237**, 2085 (1962). In each experiment counts were corrected for dilution and for a uniform thickness on the planchets. A detailed proof of structure for compounds VIII–XI is given in ref. 17 and 18.

Ic (67 mg.). To a stirred solution of the ether Ic (67 mg.) in freshly distilled propylene glycol monomethyl ether (9 ml.) and liquid ammonia (10 ml.), lithium (200 mg.) was added in several portions. The mixture was stirred for 2 hr.; then most of the ammonia was allowed to evaporate. Water was added, and the product was extracted with ether–methylene chloride (3:1). The solution was washed with water, dried, and concentrated to a residue. The sirup was dissolved in methanol (10 ml.), 3 *N* hydrochloric acid was added, and the mixture was kept at 60° for 30 min. After dilution, the product was extracted (ether–methylene chloride, 3:1), washed, and recovered by evaporation of the solvent. The residue was crystallized from acetone to yield VIa (20 mg.), m.p. 202–205° (lit.[12] 205–207°) which was acetylated (pyridine–acetic anhydride) to acetate VIb (18 mg.).

(b).—The same sequence of reactions was carried out on phenol Ia (180 mg.), prepared from trienone Vf, to yield acetate VIb (20 mg.).

The infrared spectra of all solid intermediates and the acetates VIb were indistinguishable from authentic samples.

5α,17β-Dihydroxy-1α-methyl-4-oxaestr-3-one (VII). (a).—A portion of VIb (prepared from Vd) was added to a solution of nonradioactive VIb (500 mg.) in ethyl acetate. The diluted material was recrystallized twice from ethyl acetate without change of specific activity.

A solution of diluted VIb (300 mg.) in ethyl acetate (10 ml.) was cooled to −70° and treated with a stream of ozonized oxygen freed of carbon dioxide. When the band at 242 mμ disappeared, carbon dioxide-free water (5 ml.) was added, the flask was closed with an Ascarite tube, and the mixture was frozen in liquid nitrogen. Following removal of the Ascarite tube, the flask was attached to a vacuum line, the system was evacuated, and the line was closed. The contents of the flask were thawed and distilled to dryness while the receiver was immersed in liquid nitrogen. The distillation was repeated twice more with 2-ml. portions of water. The distillate was used later to obtain carbon-4.

The nonvolatile residue was dissolved in ether–methylene chloride (3:1) and partitioned with 2 *N* sodium hydroxide. The lactol VII was recovered (200 mg.) from the alkaline solution after acidification. The lactol was recrystallized twice from ethyl acetate–hexane to yield pure VII (80 mg.).

(b).—A sample of VIb (10.5 mg.) prepared from trienone Vf was diluted with nonradioactive VIb (500 mg.) and was recrystallized twice without change of specific activity.

A portion of this diluted material (300 mg.) was treated exactly as described in part (a) to yield homogeneous VII (80 mg.).

The lactol VII showed m.p. 152–153°, λ_{max}^{MeOH} none; ν_{max}^{KBr} 3320 (sharp), 1705, 1250, 1055 cm.$^{-1}$. *Anal.* Calcd. for $C_{18}H_{28}O_4$: C, 70.10; H, 9.15. Found: C, 70.18; H, 9.00.

Carbon Dioxide from C-4.—The distillate from experiments (a) and (b) were treated in identical manner. To the thawed distillate, carbon dioxide-free 1 *N* sodium hydroxide (5 ml.) was added, and the mixture was vigorously shaken. The phases were allowed to separate, and the aqueous layer was withdrawn with a pipet. The operation was repeated twice more with 2-ml. portions of 1 *N* sodium hydroxide diluted with water (3 ml.). The combined alkaline washings were acidified with acetic acid and brought to reflux. A solution of mercuric acetate (2 g.) in carbon dioxide-free water (10 ml.) was added during 30 min., and the refluxing was continued for 60 min. The evolving gases were swept with nitrogen through a train of apparatus *via* a half-saturated barium hydroxide solution. To the barium carbonate-containing trap, perchloric acid (30%) was added, and the liberated carbon dioxide was swept with nitrogen into carbon dioxide-free 1 *N* sodium hydroxide. Then 1.1 equivalents of ammonium chloride was added, followed by a solution of barium chloride. The precipitated barium carbonate was collected by filtration (70 mg.). From experiment (b), 80 mg. of barium carbonate was obtained.

1,17β-Dihydroxy-4-methylestra-1,3,5(10)-triene (IIb).—To a sample of 4-C^{14}-androsta-1,4-diene-3,17-dione (Vd, 150 mg.) in acetic anhydride (8 ml.) a 5% solution of freshly fused zinc chloride in acetic acid (0.3 ml.) was added. The mixture was left for 24 hr. at ambient temperature, then the reaction was terminated with a large amount of ice. The steroid was recovered in the usual manner (ether–methylene chloride (3:1)). The sirup (150 mg.) was dried *in vacuo*, then ether (50 ml.) and lithium aluminum hydride (30 mg.) were added. The suspension was refluxed for 4 hr. After addition of a small amount of water to the cooled reaction mixture, the solids were separated by filtration. The filtrate was washed, dried, and concentrated to a residue. Crystallization from methanol gave IIb (85 mg.), m.p. 229–233° (lit.[8] 231–233°).

Methyl 17β-Hydroxy-2,3-bisnor-4-methyl-1,4-seco-5α-estr-4-one-1-oate (VIIIb).—A portion of the radioactive phenol IIb was diluted with nonradioactive material (3.0 g.). A sample of this material was recrystallized twice without change of specific activity.

To a solution of IIb (2.95 g.) in methanol (300 ml.) containing 2 *N* sodium hydroxide (200 ml.) at reflux (steam bath), 35% hydrogen peroxide (75 ml.) was added during 20 min. The boiling was continued for 1 hr. Most of the methanol was removed under reduced pressure, water was added, and the mixture was extracted with ether. The ether extract was not investigated. The aqueous phase was acidified with concentrated hydrochloric acid and extracted thoroughly with ether–methylene chloride (3:1). The organic phase was washed with a saturated sodium hydrogen carbonate solution (3 × 200 ml.), then with 1 *N* sodium hydroxide (3 × 200 ml.). The sodium hydroxide washings were set aside. The bicarbonate solution was acidified with concentrated hydrochloric acid to pH 1 and extracted with ether–methylene chloride (3:1). After washing with water and drying with sodium sulfate, the solution was concentrated to yield crude VIIIa[17,18] (650 mg.), m.p. 185–187°. The crude product was treated with diazomethane to provide VIIIb[17,18] (470 mg.), m.p. 129–130° after crystallization from ethyl acetate–hexane.

Methyl 5β-Acetoxy-1,5-seco-17β-trifluoroacetoxy-2,3,4-trisnorestran-1-oate (IXa).—To a stirred mixture of VIIIb (450 mg.), methylene chloride (13 ml.), and dry disodium phosphate (1 g.) at ice bath temperature, a solution of methylene chloride (10 ml.), trifluoroacetic anhydride (1.5 ml.), and 90% hydrogen peroxide (0.3 ml.) was added during 15 min. The reaction was stirred for 3 hr. at room temperature. Ice was added to decompose the reaction; then the product was dissolved in ether, washed, dried, and concentrated to a residue. The residue crystallized from ethyl acetate to yield IXa (120 mg.), m.p. 150–155° (lit.[18] 154–156°); ν_{max}^{KBr} 1780, 1730 cm.$^{-1}$.

Methyl 5β,17β-Dihydroxy-1,5-seco-2,3,4-trisnorestran-1-oate (IXb).—A solution of IXa (120 mg.) in methanol (5 ml.) and 50% sodium hydroxide (0.5 ml.) was heated at reflux for 2 hr. in an atmosphere of nitrogen. From the acidified and diluted mixture, the steroid was recovered with ether. The extract was dried and concentrated to a small volume. The solution was treated with diazomethane and concentrated to a residue. The residue crystallized from ethyl acetate to yield IXb (100 mg.), m.p. 155–159° (lit.[18] 158–159°); ν_{max}^{KBr} 3290, 1730 cm.$^{-1}$.

Saponification of the mother liquors from the Baeyer–Villiger oxidation and subsequent re-esterification with diazomethane provided additional amounts of IXb.

Methyl 1,5-Seco-2,3,4 trisnorestra-5,17-dione-1-oate (X).—Radioactive IXb (110 mg.) was diluted with nonradioactive IXb (90 mg.). The dihydroxy ester IXb (190 mg.) was treated with an oxidizing solution consisting of 90% acetic acid (10 ml.) and chromium trioxide (400 mg.). After storing the mixture for 4 hr. at room temperature, the reaction was terminated with methanol, and the products were recovered in the conventional manner. The product X crystallized from ether–hexane (140 mg.), m.p. 146–148° or 128–130° (lit.[19] 148–150°); $\nu_{max}^{CHCl_3}$ 1740, 1713 cm.$^{-1}$.

3-(1-Oxo-8β-methyl-5β-carboxymethyl-*trans*-perhydroindanyl-4α)propionic Acid (XIa).—The diketo ester X (100 mg.) was dissolved in 25% methanolic potassium hydroxide (2 ml.), and the mixture was refluxed for 1 hr. in an atmosphere of nitrogen. The product was recovered (ethyl acetate) from the diluted acidified solution. After the conventional work-up, XIa was obtained. Two recrystallizations from ethyl acetate gave homogeneous XIa (58 mg.), m.p. 157–158°, $[\alpha]^{25}D$ +89.5° (*c* 1.14 in dioxane) (lit.[19] m.p. 159–161°, $[\alpha]_D$ +92°).

The diester XIb was prepared by treatment of XIa with diazomethane. Crystallization from ether–hexane gave a sample, m.p. 91–92°; n.m.r. ($CDCl_3$) 6.63 (6H) (2-OCH_3), 8.38 τ (18-CH_3). *Anal.* Calcd. for $C_{17}H_{26}O_5$: C, 65.78; H, 8.44. Found: C, 65.78; H, 8.24.

Isolation of C-1 and C-5.—An electrolysis cell equipped with two platinum wire electrodes was charged with a mixture of the diacid IXa (29 mg.), pyridine–water (3:1, 0.3 ml.), and triethyl-

amine (0.02 ml.). A d.c. current of 12 ma. at 16 v. was passed for 1 hr. After termination of the electroly ', the evolved gases were swept with nitrogen into a half-saturated barium hydroxide solution. The obtained barium carbonate was reprecipitated as described above to yield barium carbonate (31.6 mg., 78%). In a blank experiment 0.2 mg. of residue was obtained.

The solution in the electrolysis cell was dissolved in ether and washed with dilute hydrochloric acid, 1 *N* sodium hydroxide, and water. It was then dried and concentrated to yield a viscous residue XII (7.4 mg.). A thin layer chromatograph indicated the presence of several products, and the residue was counted as such.

[CONTRIBUTION FROM THE DIVISION OF PROTEIN CHEMISTRY, INSTITUTE FOR MUSCLE DISEASE, INC., NEW YORK, N. Y.]

An Oxygen-18 Study of the Dehydration of Asparagine Amide with N,N′-Dicyclohexylcarbodiimide and *p*-Toluenesulfonyl Chloride[1]

BY D. V. KASHELIKAR[2] AND CHARLOTTE RESSLER

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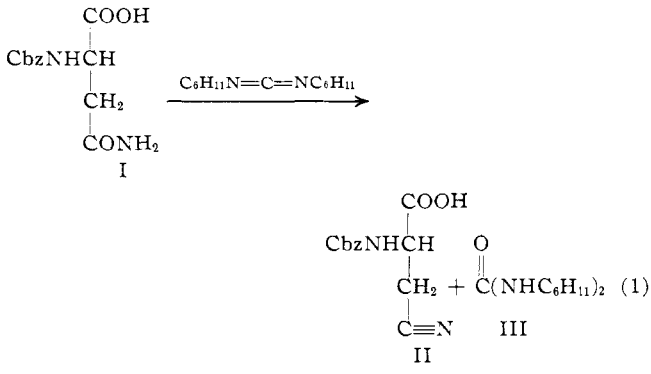
The mechanism of the dehydration of derivatives of certain amino acid amides to the corresponding amino acid nitriles with N,N′-dicyclohexylcarbodiimide in pyridine has been investigated by use of carbobenzoxy-asparagine labeled with oxygen-18 in the carboxyl group. Two suggestive routes have been considered, one of which involves formation of an activated addition intermediate between the carbodiimide and the asparagine carboxyl group, followed by intramolecular displacement of a dicyclohexylurea anion from the adduct by the *carboxamide* oxygen, succeeded by a base-catalyzed ring opening of the formed aminoisosuccinimide derivative. The second mechanism involves intramolecular interaction of the carboxylate anion with the *carboxamide* carbonyl and addition of the resulting anion at the *carboxamide* oxygen to the carbodiimide. Elimination of dicyclohexylurea from a cyclic transition state then gives rise to the common aminoisosuccinimide intermediate. The appreciable labeling of the formed N,N′-dicyclohexylurea supports the concept of an adduct between the carbodiimide and the asparagine carboxyl group. The results are consistent qualitatively with the first mentioned mechanism, although this interpretation is not considered to be unequivocal. To a smaller extent, the second mechanism, or a different one, may operate concurrently. The labeled carbobenzoxyasparagine was dehydrated also with *p*-toluenesulfonyl chloride in pyridine. The findings indicate that the reaction mechanisms with the two reagents may be similar. Syntheses of the L- and DL-isomers of carbobenzoxyasparagine labeled with oxygen-18 in the carboxyl group are presented.

Interest in the mechanism of the dehydration of asparagine amide encountered during the synthesis of certain asparagine peptides related to oxytocin[3] and vasopressin[4] has stemmed in part from the possibility of utilizing this reaction for analytical purposes in peptide chemistry, since the dehydrated asparagine residue can be easily converted to the readily identified 2,4-diaminobutyric acid.[3,5]

At the start of this study, it had been established that carbobenzoxy-L-asparagine (I) and carbobenzoxy-L-glutamine react in pyridine with the coupling agent N,N′-dicyclohexylcarbodiimide to afford in good yield N,N′-dicyclohexylurea and the ω-amino acid nitrile derivatives carbobenzoxy-β-cyano-L-alanine (II) and



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carbobenzoxy-γ-cyano-α-L-aminobutyric acid.[5] Appropriate removal of the protecting groups resulted in reasonable syntheses of the free amino acid nitriles.[5,6]

Subsequent unpublished experiments showed, however, that, when the methyl ester of carbobenzoxy-L-asparagine or nicotinamide was treated in pyridine with N,N′-dicyclohexylcarbodiimide under similar conditions, dicyclohexylurea was not formed. Moreover, evidence had accumulated from syntheses of various asparagine and glutamine peptides that peptide couplings could be effected with N,N′-dicyclohexylcarbodiimide in satisfactory yield without apparent amide dehydration in instances in which the carboxyl group that was activated belonged to an amino acid residue other than asparagine or glutamine; *i.e.*, when the asparagine or glutamine residue was not in C-terminal position.[7] These observations suggested that the carboxyl group of the asparagine or glutamine residue might be participating, perhaps intramolecularly, in the dehydration of the carboxamide group of these residues.[8a] Evidence for intramolecular group participation has been available for a number of other reactions.[9,10]

(1) Aided by Muscular Dystrophy Associations of America and by U. S. Public Health Service Grant NB 04316.

(2) Visiting Research Fellow, 1961–1962.

(3) C. Ressler, *J. Am. Chem. Soc.*, **78**, 5956 (1956).

(4) D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, *ibid.*, **78**, 5954 (1956); P. G. Katsoyannis, D. T. Gish, G. P. Hess, and V. du Vigneaud, *ibid.*, **80**, 2558 (1958).

(5) C. Ressler and H. Ratzkin, *J. Org. Chem.*, **26**, 3356 (1961).

(6) Removal of the carbobenzoxy group by hydrogenolysis in methanol with a palladium catalyst has since then been found somewhat more convenient than the procedure described, and it yields a γ-cyano-α-aminobutyric acid product that is easier to purify.

(7) An example is the synthesis of isoglutamine-oxytocin [C. Ressler and V. du Vigneaud, *J. Am. Chem. Soc.*, **79**, 4511 (1957)].

(8) (a) Since then, chiefly on the basis of similar observations that N,N′-dicyclohexylcarbodiimide fails to dehydrate asparagine ester or simple amino amide derivatives, this suggestion that the dehydration of the amide group of I is assisted by the neighboring carboxylate anion was made by B. Liberek [*Bull. Acad. Polon. Sci.*, **10**, 227 (1962)]. (b) That reaction 1 could proceed through a mechanism similar to that suggested by Cotter, Sauers, and Whelan[14] was also noted by this author.

(9) For some of these see J. Hine, "Physical Organic Chemistry," 2nd Ed., McGraw-Hill Book Co., Inc., New York, N. Y., 1962, p. 141.

(10) In the present series of compounds there is suggestive evidence that the carboxyl group of β-cyano-L-alanine can catalyze the hydrolysis of the cyano group. This amino acid is completely hydrolyzed within 1 hr. at 100° in 0.1% solution in 0.35 *N* H_2SO_4 to a mixture of asparagine and aspartic acid. In contrast, β-aminopropionitrile, as determined by paper electrophoresis, yields no detectable β-alanine and appears quite stable, as had been observed for the latter for even a longer heating period (J. T. Garbutt and F. M. Strong, Abstracts, 128th National Meeting of the American Chemical Society, Minneapolis, Minn., 1955, p. 26A.