17α-Acetoxy-3β-hydroxy-5β-methyl-A-homo-B-norpregnane-4a,20-dione (XXIX).—A. A solution of 17α-acetoxy-5α,6α-epoxy-3β-hydroxy-6β-methylpregnan-20-one (XXVI, 0.3 g.) in methylene chloride (5 ml.) was cooled to −60° and to this was added a cooled solution (−60°) of tetrahydrofuran (1.1 ml.), methylene chloride (0.5 ml.), and anhydrous hydrogen fluoride (0.8 ml.). After being kept at −5° for 5 hr., the solution was poured carefully into a saturated sodium bicarbonate solution. The organic phase was separated, and the aqueous layer was extracted with methylene chloride. The combined organic solutions were washed with water and dried. Evaporation left an amorphous residue which was dissolved in a small amount of benzene and added to a column of synthetic magnesium silicate (12 g.). Elution with 3% acetone–petroleum ether gave a crude crystalline material. Crystallization from acetone–petroleum ether yielded XXIX (118 mg.), m.p. 189–190°. This melting point was raised to 194–196° by several crystallizations, λ_{max} none; $[\alpha]^{25}D$ −30° (chloroform); ν_{max} 3350, 2940, 1730, 1695, 1445, 1370, 1255, and 1045 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{36}O_5$ (406.54): C, 71.25; H, 8.97. Found: C, 70.66; H, 9.33.

B. Boron trifluoride in ether (20 ml.) was added to a solution of XXVI (1.9 g.) in ether (100 ml.) and benzene (100 ml.). After 18 hr. the solution was neutralized with saturated sodium bicarbonate solution. The organic phase was separated, washed with water, and dried. Evaporation of the solvent afforded a gum which was dissolved in anhydrous ether and seeded with material obtained from procedure A above. In this manner a crystalline product (1.2 g.) was obtained, m.p. 174–184°. Crystallization from acetone–petroleum ether gave XXIX (0.62 g.), m.p. 195–197°, identical to the product obtained by procedure A.

17α-Acetoxy-5β-methyl-A-homo-B-norpregnane-3,4a,20-trione (XXX).—To a solution of 17α-acetoxy-3β-hydroxy-5β-methyl-A-homo-B-norpregnane-4a,20-dione (XXIX, 0.1 g.) in reagent acetone (distilled from potassium permanganate) (2 ml.) was added 8 *N* chromic acid in 8 *N* sulfuric acid dropwise until the orange color of the oxidizing agent persisted. The solution was poured into water and filtered. Two crystallizations of the solid from acetone–petroleum ether provided XXX (55 mg.), m.p. 239–240°; $[\alpha]^{25}D$ −104° (chloroform); $\lambda_{max}^{Basic\ MeOH}$ 300 mμ (ε 18,100); ν_{max} 1740, 1720, 1700, 1262, and 1248 cm.$^{-1}$.

Anal. Calcd. for $C_{24}H_{38}O_5$ (402.51): C, 71.61; H, 8.51. Found: C, 71.46; H, 8.64.

17α-Hydroxy-5β-methyl-A-homo B-norpregnane-3,4a,20-trione (XXXI).—5α,6α - Epoxy - 3,20 - bisethylenedioxy - 6β - methylpregnan - 17α - ol (XXIII, 0.2 g.) was suspended in acetone (8 ml.) and 72% perchloric acid (2 drops) was added. Solution was effected immediately. The mixture after 2 hr. at room temperature was treated with dilute sodium bicarbonate solution, and the solid which separated was collected by filtration and washed with water. This material was crystallized several times from acetone–water to give XXXI (30 mg.), m.p. 194–196°; $[\alpha]^{25}D$ −124° (chloroform); $\lambda_{max}^{Basic\ MeOH}$ 300 mμ (ε 18,600); ν_{max} 3460, 2970, 1720, 1695, 1390, 1355, 1200, and 1080 cm.$^{-1}$.

Anal. Calcd. for $C_{22}H_{32}O_4$ (360.48): C, 73.30; H, 8.95. Found: C, 73.17; H, 9.33.

Synthesis of α-Amino-γ-hydroxy Acids: γ,γ′-Dihydroxyvaline

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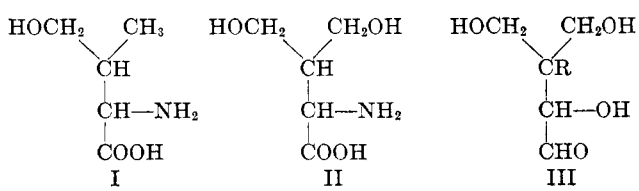
γ-Hydroxyvaline (I) and γ,γ′-dihydroxyvaline (II) have been prepared by a modified Erlenmeyer synthesis. The geometry of the intermediate azlactones IVb and IVc and of the corresponding benzoylamino acrylic esters VIIb and VIIc has been determined.

Until a few years ago, homoserine was the only α-amino-γ-hydroxy acid known with certainty to occur in Nature. Recently, however, a prodigious number of such compounds has been detected, both in free form and as peptide constituents and their chemistry has been the subject of considerable study.[1]

We wish to describe a simple synthetic method leading to α-amino acids with chain branching in the beta position and carrying hydroxyl groups in one or both of the gamma positions. The method offers an alternate synthesis for γ-hydroxyvaline (I), recently isolated from crowngall tumors of *Kalanchoe daigremontiana* and synthesized from α-chloro-β-methyl-γ-butyrolactone.[2] More importantly, however, it has permitted us to prepare a new, otherwise difficulty accessible amino acid, γ,γ′-dihydroxyvaline (II). Although this compound has not been found to occur naturally, closely related structures like the sugars cordicepose and apiose (III. R = H and OH respectively) have been isolated,[3] and the role of γ,γ′-dihydroxyvaline itself as a possible biogenetic precursor of the antibiotic Cephalosporin C has been discussed.[4] The method of synthesis is based on the observation that the Erlenmeyer azlactone synthesis, although seldom practicable with ketones,[5] can be successfully extended to the acetates of α-hydroxy and α,α′-dihydroxy ketones by applying the modified conditions of Baltazzi and Robinson.[5c] Thus, by using equimolecular amounts of ketone and hippuric acid, three moles of acetic anhydride, lead(II) acetate as a base, and tetrahydrofuran as the solvent, the azlactones IV are formed in practical yields, readily isolable by crystallization.[6]



The exocyclic double bond in compounds IV can be hydrogenated (palladium–charcoal, dioxane) to give the "dihydro," azlactones V with little or no hydrogenolysis of the allylic acetate groups and the azlactones hydrolyzed with hydrochloric acid to the aminohydroxy acid lactone hydrochlorides VI, which can then be converted to the free amino acids by treatment with ammonia.

(1) For reviews, see Th. Wieland, *Angew. Chem.*, **72**, 892 (1960); H. Musso, *ibid.*, **68**, 313 (1956); A. I. Virtanen, *ibid.*, **67**, 381 (1955).

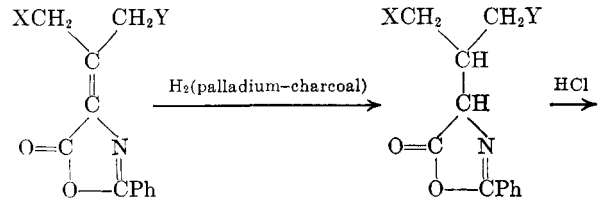
(2) J. K. Pollard, E. Sondheimer, and F. C. Steward, *Nature*, **182**, 1356 (1958).

(3) W. G. Overend, M. Stacey in "Advances in Carbohydrate Chemistry," Vol. 8, Academic Press Inc., New York, N. Y., 1953, p. 52; C. S. Hudson, *ibid.*, Vol. 4, 1949, p. 57.

(4) E. P. Abraham and G. F. Newton, *Biochem. J.*, **79**, 377 (1961).

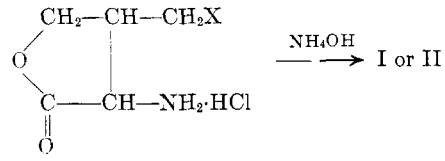
(5) (a) H. E. Carter, *Org. Reactions*, **III**, 206 (1946); (b) J. W. Cornforth in "Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 730 ff; (c) E. Baltazzi and R. Robinson, *Chem. Ind.* (London), 191 (1954).

(6) Substitution of 2-phenyl-2-oxazolin-5-one [J. M. Stewart and D. W. Woolley, *J. Am. Chem. Soc.*, **78**, 5336 (1956)] for hippuric acid did not improve the yields.

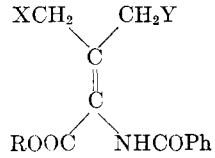


IVa. X = Y = H
IVb. X = H, Y = AcO
IVc. X = AcO, Y = H
IVd. X = Y = AcO

V. X = H or AcO
Y = H or AcO



VIa. X = H
VIb. X = OH



VIIa. X = Y = H, R = CH_3
VIIb. X = H, Y = AcO, R = CH_3
VIIc. X = AcO, Y = H, R = CH_3
VIId. X = Y = AcO, R = CH_3
VIIe. X = MeO, Y = AcO, R = CH_3
VIIf. X = Y = AcO, R = C_2H_5
VIIg. X = Y = AcO, R = H

It is interesting to note that in contrast to the azlactones IV, the substituted acrylic acids and esters VII undergo only hydrogenolysis to give the known α-benzamido-β,β-dimethylacrylic acid (ester VIIa), the double bond of which resists hydrogenation under the conditions used.

In the condensation of acetol acetate with hippuric acid both geometrical isomers IVb and IVc are formed and can be separated by fractional crystallization. Their geometry will be discussed below. On catalytic hydrogenation both IVb and IVc yield what appear to be identical mixtures of the diastereomeric dihydro azlactones V (X = H, Y = AcO), and the γ-hydroxyvaline I obtained on hydrolysis of V is likewise a mixture of diastereomers similar in melting point to the product described by Pollard, Sondheimer, and Steward.[2]

The new amino acid, γ,γ′-dihydroxyvaline (II), obtained on acid hydrolysis of the azlactone V (X = Y = AcO) followed by ion exchange chromatography, crystallized readily as the lactone hydrochloride VI (X = OH), which could be converted with ammonia into the likewise crystalline D,L-amino acid. Resolution of the latter was achieved by incubating the lithium salt of its *N*-chloroacetyl derivative with hog kidney acylase and separating the dechloroacetylated L-acid from the *N*-chloroacetyl-D-derivative by ion exchange methods. The specific rotations observed were $[\alpha]^{24}D$ −12.2° for the L-acid and +13.7° for the D acid (c = 1–2, freshly prepared solutions in 0.1 *N* potassium bicarbonate). Table I contains paper chromatographic data obtained in three systems.

The precise geometry of the *cis-trans* isomeric azlactones IVb and IVc and of the acrylic esters VIIb and VIIc has been determined by n.m.r. spectroscopy on the basis of the elegant work of Jackman.[7] Comparing the position of the C-methyl signals in the acrylic ester VIIa

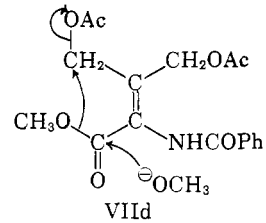
TABLE I

$R_{alanine}$-VALUES FOR γ-HYDROXYVALINE AND γ,γ′-DIHYDROXYVALINE[a]

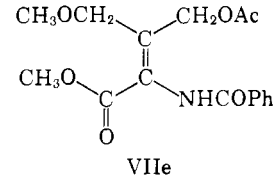
	n-BuOH–AcOH–H_2O 4:1:1	65% pyridine	95% ethanol
γ-Hydroxyvaline	0.95	1.21	1.36
γ-Hydroxyvaline lactone			2.72
γ,γ′-Dihydroxyvaline	0.55	1.10	0.69
γ,γ′-Dihydroxyvaline lactone	1.18	1.46	2.64

[a] Descending, Whatman paper no. 1, solvent front traveled 40 cm. Spots detected by ninhydrin.

with those of methyl β,β-dimethylacrylate (Table II) we find almost complete correspondence indicating that the α-benzoylamino group exerts no greater effect on the C-methyl groups than the α-proton. This confirms the view readily gained from inspection of molecular models that the most stable conformations are those in which the benzoyl group points away from the *cis*-β-methyl group. Since the C-methyl signal in methyl β,β-dimethylacrylate appearing at lower field (τ = 7.88) has been assigned to the more shielded methyl group *cis* to the carbomethoxy group, the same assignment is made in the case of VIIa. For similar reasons, the methylene protons of the β-acetoxymethylene group in VIId appearing at lower field are assigned the *cis*, those at higher field the *trans* structure with respect to the carbomethoxy group. Inspection of the τ values for the methyl and methylene group in VIIb and VIIc now permits the assignments shown in Table II. The agreement between the signals of each of the two methyl groups in VIIa and the corresponding signals in VIIb and VIIc are particularly striking. Chemical confirmation of the above assignments was possible with the aid of a product obtained from IVd by brief heating in methanol in the presence of potassium acetate. The structure VIIe assigned to this compound is most satisfactorily rationalized as arising from the intermediate VIId by an intramolecular substitution reac-



VIId



VIIe

tion involving the carbomethoxy and acetoxymethylene groups *cis* to each other. The n.m.r. spectrum, as expected, shows the *trans*-acetoxymethylene group at higher field and a new signal (τ = 5.83) for the *cis*-methoxymethylene group.

The spectra of the corresponding azlactones IVa–IVd do not show the pronounced differences for the *cis* and *trans* methyl and methylene signals seen with the acrylic esters VIIa–VIId, although the differences are in the same direction. Moreover, these signals appear at lower field. This is ascribed to greater shielding of these protons due to the cisoid nature of the α,β-unsaturated carbonyl system in the azlactones—the car-

(7) L. M. Jackman: "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, New York, N. Y., 1959, pp. 58, 121.

TABLE II

METHYLENE AND METHYL PROTON MAGNETIC RESONANCE SIGNALS OF AZLACTONES IV AND SUBSTITUTED ACRYLIC ESTERS VII[a]

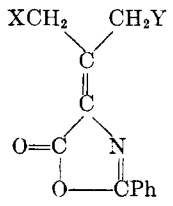
	$AcOCH_2-\overset{\vert}{C}=$		$MeOCH_2-\overset{\vert}{C}=$	$-COOCH_3$	$CH_3OCH_2-\overset{\vert}{C}=$	CH_3COO-		$CH_3-\overset{\vert}{C}=$	
IVa								7.61	7.67
IVb		4.76				7.85		7.60	
IVc	4.65						7.90		7.67
IVd	4.60	4.70				7.85	7.90		
Methyl β,β-dimethyl-acrylate				6.30				7.88	8.16
VIIa				6.30				7.85	8.15
VIIb		5.35		6.20			7.95	7.86	
VIIc	4.88			6.22		7.90			8.10
VIId	5.05	5.26		6.15		7.85	7.90		
VIIe		5.22	5.83	6.15	6.62		7.84		

[a] Spectra taken at 60 Mc. (Varian A-60 n.m.r. spectrometer) in deuteriochloroform solutions with tetramethylsilane as internal standard. Signal positions are given in τ values.

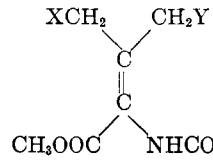
TABLE III

ULTRAVIOLET ABSORPTION SPECTRA OF SUBSTITUTED AZLACTONES[a] AND METHYL BENZAMIDO ACRYLATES[b]

(Absorption maxima, mμ; log ε values in brackets)



X = Y = H	Sh 321 (4.17)	310 (4.38)	298 (4.37)	244 (4.08)	235 (4.08)	Sh 232 (4.06)	224 (4.04)
X = H, Y = AcO	Sh 323 (4.19)	306 (4.28)		244 (4.07)	236 (4.07)	Sh 230 (4.03)	225 (4.00)
X = AcO, Y = H	Sh 323 (4.21)	306 (4.37)		245 (4.06)	237 (4.07)	Sh 232 (4.05)	
X = Y = AcO		308 (4.29)		245 (3.99)	237 (4.03)		



X = Y = H	226 (4.15)		
X = H, Y = AcO	231 (4.20)	Sh 250	
X = AcO, Y = H	230 (4.23)		
X = Y = AcO	244 (4.13)	235 (4.15)	
X = MeO, Y = AcO	251 (4.14)	233 (4.17)	

[a] In cyclohexane solution. [b] In ethanol solution.

bonyl group being directed towards these protons—as compared to the *s-trans* conformation prevalent in the acrylic esters, and to the closeness of the anisotropic N=C grouping in the azlactones in place of the benzoylamino group in the esters.

The ultraviolet absorption maxima of the azlactones IV and esters VII are summarized in Table III. While no correlation with the geometrical isomerism can be made, attention is drawn to the increasing simplicity of the azlactone spectra with the increasing number of acetoxy substituents, which may be related to the strongly decreased ability of the acetoxymethylene groups to enter into hyperconjugation with the extended conjugated system, thereby increasing the number of relatively low energy excited states.

Experimental

2-Phenyl-4-(2-acetoxy-1-methylethylidene)-2-oxazolin-5-one (IVb and IVc).—A mixture of 35.90 g. of hippuric acid (0.20 mole), 27.80 g. of acetol acetate (0.24 mole), and 32.50 g. of anhydrous lead(II) acetate (0.10 mole) in 60.10 g. of acetic anhydride (0.60 mole) and 460 ml. of peroxide free tetrahydrofuran was heated under reflux for 16 hr. in a nitrogen atmosphere. After cooling, the mixture was filtered and evaporated to dryness *in vacuo*. The residue was taken up in 700 ml. of benzene and treated with hydrogen sulfide for 5 min. at 10°. The filtered solution was again evaporated to a slowly crystallizing mixture of the geometrical isomers IVb and IVc (40.32 g. or 78%). After short boiling with 50 ml. of isopropyl alcohol, 11.32 g. (22%) of isomer IVb crystallized (m.p. 98–101°) in the form of yellow needles. The analytical sample, obtained after three recrystallizations from diisopropyl ether, melted at 101–102°.

Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.77; H, 5.21; N, 5.14.

The isopropyl alcohol mother liquor of IVb was concentrated *in vacuo* until it weighed 53 g. On cooling, 5.31 g. (10%) of isomer IVc separated (m.p. 76–79°). Two recrystallizations from diisopropyl ether yielded the pure compound in form of yellow needles: m.p. 81.5–82°. Mixed m.p. with IVb: 66–90°.

Anal. Calcd. for $C_{14}H_{13}NO_4$: C, 64.86; H, 5.05; N, 5.40. Found: C, 64.91; H, 5.08; N, 5.62.

The infrared absorption spectra of the isomers (KBr) showed differences in the fingerprint region and had the following more important common bands: IVb: 5.57 (oxazolone C=O), 5.76, 8.01 (acetate) and 5.97 μ (oxazolone C=N); IVc: 5.60, 5.74, 8.03 and 5.98 μ.

γ-Hydroxyvaline (I).—To a solution of 825 mg. (3.18 mmoles) of 2-phenyl-4-(2-acetoxy-1-methylethylidene)-2-oxazolin-5-one (IVb, m.p. 100–101°) in 15 ml. of peroxide free dioxane there was added 225 mg. of prehydrogenated palladium-on-charcoal (5%) catalyst and the mixture was stirred in hydrogen atmosphere at room temperature. After the uptake of 1 mole of hydrogen (135 min., 80 ml. at 21°/760 mm. saturated with dioxane), the hydrogenation was stopped and the filtered solution evaporated to give 915 mg. of a pale yellow oil. The infrared absorption spectrum (neat) indicated the formation of the "dihydro" azlactone V (5.47 μ, oxazolone C=O; 5.74, 8.13 μ, acetate) and the presence of some unhydrogenated "unsaturated" azlactone (weak band at 5.58 μ). Hydrogenation of the isomer IVc proceeded in the same way and yielded an oil with its infrared absorption spectrum indistinguishable from that described above.

The crude, oily **V** (860 mg.) was heated under reflux with a mixture of 6 ml. of concentrated hydrochloric acid and 4 ml. of water for 3.5 hr. and left in the refrigerator (+8°) overnight. The mixture was then filtered from 345 mg. (94%) of benzoic acid and evaporated to dryness *in vacuo*. The residual glass (488 mg.) was dissolved in 5 ml. of water and passed through a column of Amberlite IR 120 (H^+ form, 8 ml. resin). The column was washed with 50 ml. of water. The amino acid was obtained by

elution with 15 ml. of 1.5 *N* aqueous ammonia, boiling the eluate until the ammonia had escaped, and by evaporation of the aqueous solution *in vacuo*. The white crystalline residue (303 mg. 76%, m.p. 206–209°) was recrystallized for analysis from water–ethanol (m.p. 208–209° dec., reported[2] 209–211°).[8]

Anal. Calcd. for $C_5H_{11}O_3N$: C, 45.10; H, 8.33; N, 10.52. Found: C, 45.17; H, 8.50; N, 10.55.

2-Phenyl-4-(2-acetoxy-1-acetoxymethylethylidene)-2-oxazolin-5-one (IVd). (a)—A mixture of 5.37 g. of hippuric acid (30 mmoles), 6.25 g. of diacetoxyacetone (prepared from dihydroxyacetone according to H. O. L. Fischer and L. Feldmann[9]), 4.86 g. (15 mmoles) of anhydrous lead(II) acetate, 9.20 g. (90 mmoles) of acetic anhydride, and 100 ml. of peroxide free tetrahydrofuran was stirred and heated under reflux for 20 hr. in an atmosphere of nitrogen. The filtered mixture was then evaporated *in vacuo*, and the residue dissolved in 50 ml. of benzene and treated with hydrogen sulfide for 5 min. at 10°. The filtered red solution on evaporation gave 11.89 g. of a slowly crystallizing oil, from which, on trituration with 30 ml. of *t*-butyl alcohol, 4.02 g. (41.4%) of the oxazolone IVd could be separated (m.p. 125–128°). Two recrystallizations from *t*-butyl alcohol and benzene–hexane, respectively, raised the melting point to 130.5–131° (yellow needles); λ_{max}^{KBr} 5.56 μ (oxazolone C=O); 5.75, 8.00–8.10 μ (acetates); 5.94 μ (oxazolone C=N).

(b) A mixture of 18.75 g. of diacetoxyacetone[9] (108 mmoles), 14.50 g. of 2-phenyl-2-oxazolin-5-one[7] (90 mmoles), 13.20 g. lead(II) acetate (40.5 mmoles), 20 ml. of acetic anhydride (181 mmoles), and 300 ml. of peroxide free tetrahydrofuran gave, after 12 hr. refluxing in nitrogen atmosphere and a work-up similar to that described under **a**, 10.86 g. (38%) of the azlactone (IVd) with m.p. 129–130°.

DL-γ,γ′-Dihydroxyvaline (II).—A solution of 6.30 g. (20 mmoles) of 2-phenyl-4-(2-acetoxy-1-acetoxymethylethylidene)-2-oxazolin-5-one (IVd) in 90 ml. of peroxide free dioxane was hydrogenated in a Parr hydrogenation apparatus at room temperature in the presence of 0.30 g. of 5% palladium on carbon catalyst. The hydrogenation was interrupted after uptake of 20 mmoles of hydrogen (54 min.) and the filtered solution evaporated to give 6.27 g. of an oil. On treatment with 6.0 ml. of cold isopropyl alcohol, 0.65 g. of starting material crystallized and was removed by filtration. The oil obtained by evaporation of the mother liquor (5.45 g.) consisted of the "dihydro" azlactone (V. X = Y = AcO), as indicated by its infrared absorption spectrum: bands at 5.44 μ ("dihydro" azlactone C=O), 5.70, 8.10–8.15 μ (acetates), 6.03 μ ("dihydro" azlactone C = N). Bands characteristic for the starting material (5.56, 5.94 μ) were virtually absent.

A solution of 5.44 g. of the above oil (17 mmoles) was heated under reflux with 28 ml. of 5 *N* hydrochloric acid for 20 hr. On cooling, 1.87 g. (90%) of benzoic acid crystallized and was removed by filtration. The filtrate was evaporated *in vacuo* to remove hydrochloric acid and then redissolved in 50 ml. of water. The solution was passed through a 9-ml. column of Amberlite IR 120 resin (H^+ form, 1.9 meq./ml.) which was subsequently washed with 300 ml. of water and finally eluted with 100 ml. of 1.5 *N* aqueous ammonia. Evaporation of the ammonia eluate yielded 1.29 g. of a dark oil, which was heated with 7.5 ml. of 5 *N* hydrochloric acid and 0.50 g. of Darco for 10 min. The filtered solution gave on evaporation 1.81 g. (57%) of the diastereomers of **γ,γ′-dihydroxyvaline lactone hydrochloride** (VI. X = OH). Recrystallization from 36 ml. of ethanol–ethyl acetate 2:1 gave 0.82 g. of white needles, m.p. 192–195° dec.

Anal. Calcd. for $C_5H_9NO_3 \cdot HCl$: C, 35.83; H, 6.01; N, 8.36; Cl, 21.16. Found: C, 35.92; H, 6.09; N, 8.24; Cl, 21.59.

A second crop (0.26 g., m.p. 176–184°) crystallized on standing from the mother liquor. The infrared absorption spectra showed two sharply resolved lactone absorption bands λ_{max}^{Nujol} 5.54, 5.60 μ.

A solution of 830 mg. (5.30 mmoles) of the lactone hydrochloride in 150 ml. of water was passed through a weakly basic anion exchange column (40 ml., Amberlite IR 4B, OH^- form, 2.5 meq./ml.) in 3 hr. The column was washed with 50 ml. of water and to the combined solutions there was added 50 ml. of concentrated aqueous ammonia. After boiling for 1 hr., the solution was evaporated to dryness *in vacuo* to yield 490 mg. (62%) of an oil, which solidified on treatment with aqueous acetone to white crystals of m.p. 160–168°. After two recrystallizations from water–acetone (1:3 v./v.) the pure **DL-γ,γ′-dihydroxyvaline** (306.6 mg., 39%) melted at 169–170°.

Anal. Calcd. for $C_5H_{11}NO_4$: C, 40.26; H, 7.43; N, 9.39. Found: C, 40.24; H, 7.43; N, 9.24.

Resolution of DL-γ,γ′-Dihydroxyvaline.—A solution of 3.035 g. (18.1 mmoles) of DL-γ,γ′-dihydroxyvaline lactone hydrochloride (VI. X = OH) in 25 ml. of water was placed in a Beckman Automatic Titrator (Model K), in which an efficient vibration mixer replaced the stirrer. Upon setting the pH dial to 8.5, the titrator added 5.30 ml. of 3.56 *N* sodium hydroxide solution (18.8 mmoles, NaOH). The solution was then cooled and, under strong vibration, 6.20 g. (36.2 mmoles) of chloroacetic anhydride was added in ten equal portions over a period of 1 hr. Simultaneously, the titrator added 15.25 ml. of 3.56 *N* sodium hydroxide solution (54.3 mmoles, NaOH). After one more hour of vibration at room temperature, the solution was acidified to pH 1.5 by the addition of 4.0 ml. of concentrated hydrochloric acid and extracted with ethyl acetate (twelve 10-ml. portions). Evaporation of the dried ethyl acetate solution yielded an oil, from which most of the chloroacetic acid was eliminated by successive extractions with hot hexane (five 10-ml. portions) and high vacuum drying over solid potassium hydroxide. The residue consisted of 2.55 g. (50%) of crude **α-chloroacetamino-β-chloroacetoxymethyl-γ-butyrolactone**, which remained a viscous oil. λ_{max}^{neat} 2.92, 5.63–5.70, 5.99, 6.48, 6.52 and 8.45 μ.

Anal. Calcd. for $C_9H_{11}NO_5Cl_2$: C, 38.04; H, 3.90; Cl, 24.95; N, 4.93. Found: C, 38.64; H, 4.66; Cl, 22.93; N, 4.64.

A 2.37-g. sample (8.4 mmoles) of the above oil was vibrated in 50 ml. of water and by setting the pH dial of the autotitrator to 9.0, an 1.835 *N* lithium hydroxide solution was automatically added. The base uptake virtually stopped after addition of 8.30 ml. (15.3 mmoles LiOH; 180 min. at room temperature) and a clear solution resulted. After dilution to 180 ml. and adjusting the pH to 8.0, 60 mg. of hog kidney acylase powder (Nutrition Biochemical Corporation, Cleveland, Ohio) was added and the mixture kept at 38° ± 1° for 90 hr. The enzyme was then eliminated by stirring the solution (pH 6) with 1.0 g. of "Darco" for 1 hr. at room temperature and the filtered solution was evaporated *in vacuo* to yield 2.36 g. of an oil. The latter was redissolved in 25 ml. of water and passed through a column of Amberlite IR 120 cation exchange resin (H^+ form, 1.9 meq./ml., 20 ml.). The column was washed with 250 ml. of water and the combined solutions were evaporated *in vacuo* to yield 1.417 g. of an oil, from which the **D-γ,γ′-dihydroxyvaline** was obtained after hydrolysis with boiling 5 *N* hydrochloric acid (25 ml., 20 hr.) followed by adsorption on Amberlite IR 120 resin (20 ml. H^+ form) and elution with 1.5 *N* aqueous ammonia. Evaporation of the ammonia eluate gave 240 mg. (39% based on the dichloroacetyl lactone) of an oil, which soon solidified. After one recrystallization from water–acetone 1:3 v./v., colorless needles of m.p. 168–172° were obtained.

The **L-γ,γ′-dihydroxyvaline** was eluted from the first column with 1.5 *N* aqueous ammonia (250 ml.). The 454 mg. of oily product, obtained on evaporation, solidified on treatment with acetone. Recrystallization from water–acetone 1:3 yielded colorless needles, m.p. 174.5–175°.

The specific rotations of the D and L acids, measured on freshly prepared solutions in 0.1 *N* potassium hydrogen carbonate, were $[\alpha]_D$ +13.7° and $[\alpha]_D$ −12.2°, respectively (*c* was 1.90 and 1.49 respectively).

Methyl α-Benzamido-β-acetoxymethylisocrotonate (VIIb).—To a solution of 120.4 mg. of the azlactone IVb in 10 ml. of anhydrous methanol was added 0.01 ml. of concentrated sulfuric acid. After 30 min. heating under reflux, the cooled mixture was poured onto 25 ml. of ice cold 2% sodium bicarbonate solution and extracted with chloroform. The solid obtained after evaporation of the chloroform solution (97.7 mg., m.p. 106.5–109) was twice recrystallized from methylene chloride–hexane to yield needles of m.p. 110–111°. λ_{max}^{KBr} 3.05, 5.76, 5.98, 6.57, 7.50, and 8.10 μ.

Anal. Calcd. for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81; methoxy, 10.63. Found: C, 62.07; H, 5.53; N, 4.71; methoxy, 10.50.

Methyl α-Benzamido-β-acetoxymethylcrotonate (VIIc).—A solution of 184.9 mg. of the azlactone IVc in 10 ml. of anhydrous methanol was treated with 0.01 ml. of concentrated sulfuric acid and heated under reflux for 30 min. The mixture was then poured into 25 ml. of ice cold 2% sodium bicarbonate solution and

(8) The γ-hydroxyvaline thus obtained contained traces of valine, as revealed by paper chromatography. In ref. 2, a simple ion exchange process for the purification of hydroxyvaline is described, which can be applied here if the presence of traces of valine is disturbing.

(9) H. O. L. Fischer and L. Feldmann, *Ber.*, **62B**, 854 (1929).

extracted with chloroform. Evaporation of the chloroform solution yielded 187.7 mg. of solid material, which after three recrystallizations from methylene chloride–diisopropyl ether furnished 102 mg. of white needles of m.p. 106.5–107°. Sublimation at 105°/0.003 mm. raised the m.p. to 109.5–110°. Mixed m.p. with VIIb: 85–100°. λ_{max}^{KBr} 3.07, 5.71, 5.78, 6.06, 6.59, 7.60, and 8.13 μ.

Anal. Calcd. for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81; methoxy, 10.63. Found: C, 61.61; H, 6.04; N, 4.85; methoxy, 10.92.

Methyl α-Benzamido-β,β-di-acetoxymethylacrylate (VIId).—A solution of 4.05 g. (12.75 mmoles) of the azlactone IVd in 100 ml. of anhydrous methanol was heated under reflux for 48 hr. Evaporation gave an oil (4.30 g.), from which 2.29 g. (52%) of crystals of m.p. 97–102° could be separated by trituration with ether. Recrystallization from methylene chloride–diisopropyl ether raised the m.p. to 105–106°. λ_{max}^{KBr} 3.08, 5.73–5.76, 6.04, 6.59, and 7.50 μ.

Anal. Calcd. for $C_{17}H_{19}NO_7$: C, 58.45; H, 5.48; N, 4.01; methoxy, 8.88; acetyl, 24.60. Found: C, 58.45; H, 5.77; N, 4.00; methoxy, 9.14; acetyl, 25.18.

The ethanolysis product (VIIf) obtained in an analogous manner, had a melting point of 106.5–107°.

α-Benzamido-β,β-diacetoxymethylacrylic Acid (VIIg).—A solution of 500 mg. (1.58 mmoles) of the azlactone IVd in 20 ml. of 50% aqueous dioxane was heated under reflux for 30 min. On evaporation, a crystalline residue was obtained which on recrystallization from water yielded 465 mg. of the acid (m.p. 129–130°). The analytical sample obtained after two recrystallizations from ethyl acetate–hexane melted at 135–135.5°. λ_{max}^{KBr} 2.06, 3.35–3.85, 5.72, 5.86, 6.00, 6.05, 6.63, 6.72, 8.06, and 8.23 μ.

Methyl α-Benzamido-β-acetoxymethyl-γ-methoxyisocrotonate (VIIe).—A mixture of 3.17 g. (10.0 mmoles) of the azlactone IVd and 0.98 g. (10.0 mmoles) of anhydrous potassium acetate in 50 ml. of anhydrous methanol was heated under reflux for 30 min. The residue obtained after evaporation *in vacuo* was thoroughly extracted with benzene to yield 3.56 g. of a light yellow oil, which was subsequently chromatographed on 158 g. of neutral alumina (Woelm, activity II). The first oily fractions obtained on elution with benzene and benzene–ether (9:1) solidified on standing (510 mg., m.p. 88–96°) and gave on recrystallization from chloroform–hexane white needles of m.p. 100–101°. λ_{max}^{Nujol} 3.05, 5.76, 5.83, 5.99, 6.07, 6.60, 6.74, 7.53, 8.10, 8.20, 9.25, and 9.35 μ.

Anal. Calcd. for $C_{16}H_{19}NO_6$: C, 59.80; H, 5.96; N, 4.36; methoxy, 19.30. Found: C, 59.61; H, 5.69; N, 4.27; methoxy, 19.08.

Elution of the column with chloroform–methanol (9:1) yielded 1.013 g. (84%) of benzamide of m.p. 115–120°.

Methyl α-Benzamido-β,β-dimethylacrylate (VIIa).—A solution of 151.5 mg. (0.435 mmole) of methyl α-benzamido-β,β-di-acetoxymethylacrylate (VIId) in 5 ml. of methanol was stirred with 50 mg. of prehydrogenated 5% palladium–charcoal catalyst (Baker) in an atmosphere of hydrogen. After an uptake of 0.885 mmoles of hydrogen (15 min. at room temperature), no more gas was consumed. Evaporation of the filtered solution gave 103.2 mg. (100%) of a solid, which on sublimation at 110°/0.005 mm yielded crystals of m.p. 137–137.5°, undepressed by an authentic specimen[5] of methyl α-benzamido-β,β-dimethylacrylate. The infrared absorption spectra (KBr pellets) were superimposable. Similar results were obtained when dioxane was used as a solvent, with the exception that hydrogenation proceeded more slowly.

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Lactones Derived from 3-(17β-Hydroxysteroid-16β-yl)propionic Acids

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A series of steroid delta lactones was prepared for comparison with the previously described gamma lactones. The 3β,17β-diacetoxy-5α-androstan-16β-ylacetic acid, the corresponding 5-unsaturated compound and 3,17β-diacetoxy-1,3,5(10)-estratrien-16β-ylacetic acid were converted by the Arndt–Eistert method into the corresponding propionic acids, which in turn yielded the desired lactones.

As a part of a continuing study of ring D modified steroidal hormones, we are reporting several new lactones having a three-carbon side chain structure at position 16 of the androstane and estrane nuclei. The 16β-acetic acids of the androstane[1] and estrane[2] series described earlier served as intermediates in this work.

The 3β,17β-diacetoxy-5α-androstan-16β-ylacetic acid (I)[1] was converted to its acid chloride II, which in turn was allowed to react with diazomethane to yield the diazo ketone III. The treatment of III with silver oxide in methanolic solution led to the isolation of methyl 3-(3β,17β-diacetoxy-5α-androstan-16β-yl)propionate (IV) in variable yields. The alkaline hydrolysis of the latter (IV) and subsequent acidification of the reaction mixture gave the dihydroxy acid V which was converted to 3-(3β,17β-dihydroxy-5α-androstan-16β-yl)propionic acid lactone (VI) at elevated temperature. The lactone VI was further characterized as the 3-acetate VII.

In order to overcome the erratic yields first encountered in the Arndt–Eistert rearrangement of the diazo ketone III, the latter was treated in benzyl alcohol and 2,4,6-trimethylpyridine at elevated temperature.[3] The resulting intermediate was hydrolyzed and finally subjected to pyrolysis to give the desired lactone VI in reproducible yields.

When VI was oxidized in a two-phase system,[4] 3-(17β-hydroxy-3-oxo-5α-androstan-16β-yl)propionic acid lactone (VIII) was obtained. From the latter (VIII) 3-(17β-hydroxy-3-oxo-1,4-androstadien-16β-yl) propionic acid lactone (IX) was prepared by bromination followed by the elimination of the elements of hydrogen bromide.[5]

In a similar manner the available 3β,17β-diacetoxy-5-androsten-16β-ylacetic acid (X)[1] was converted to the acid chloride XI, which gave rise to the diazo ketone XII. This intermediate (XII) was subjected to the Arndt–Eistert rearrangement under the conditions recommended by Wilds and Meader[3] and the crude inter-

(1) P. Kurath and W. Cole, *J. Org. Chem.*, **26**, 1939 (1961).
(2) P. Kurath and W. Cole, *ibid.*, **26**, 4592 (1961).
(3) A. L. Wilds and A. L. Meader, Jr., *J. Org. Chem.*, **13**, 763 (1948). We wish to express our thanks to Dr. C. Hummel Winestock for calling attention to this work.
(4) W. F. Bruce, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, New York, N. Y., 1943, p. 139.
(5) R. Joly, J. Warnant, G. Nominé, and D. Bertin, *Bull. soc. chim. France*, 366 (1958). P. Wieland, K. Heusler, and A. Wettstein, *Helv. Chim. Acta*, **43**, 523 (1960).