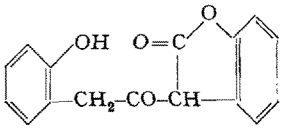
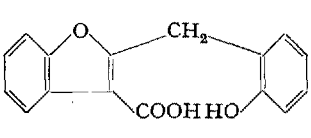
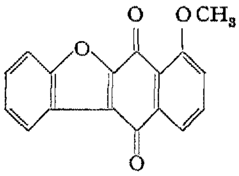
coumaranone-2 (I). On boiling this compound with hydrochloric acid in acetic acid, the furan ring is opened up and reconstituted to give an isomeric phenolic acid (m. p. 194–95°; methyl ester, m. p. 131°) whose structure is found to be (II). The methyl ether (m. p. 157·5°) of this acid on cyclisation by way of the acid chloride,



I



II



III

afforded 7-methoxy-11-hydroxy-β-brazan (not isolated) which furnished the corresponding quinone (III, m. p. 242°) on oxidation with chromic acid in acetic acid. On energetic treatment with hydriodic acid, this quinone gave a small quantity of β-brazan[3] (m. p. 208°) identical with an authentic specimen.

J. N. Chatterjea

Chemical Laboratory, Science College, Patna-5, India, June 9, 1956.

Résumé

L'autocondensation de l'isocoumaranone donne le composé (I), qui par l'acide hydrochlorique est isomerisé en l'acide (II). Celui-ci est transformé en β-brazan par cinq réactions consécutives.

[3] J. N. Chatterjea, J. Indian chem. Soc. *31*, 101 (1954).

The Application of Hudson's Lactone Rule to γ- and δ-Hydroxyamino Acids and the Question of the Configuration of δ-Hydroxy-L-lysine from Collagen

Configurational assignments for L-*threo*- and L-*erythro*-γ-hydroxyornithine (IIa; Va) and for their corresponding *cis*- and *trans*-disubstituted γ-lactones (IIIa; IVa) have been made on the basis of their conversions to hydroxyprolines[1].

In a greatly simplified presentation of the complicated experimental results (complete retention of configuration at C [2]) IIa leads to allohydroxy-L-proline (Ia) and Va to hydroxy-L-proline (VI)[1].

The lactone of N-carbobenzyloxy-γ-allohydroxy-L-proline (VIIb)[2] has been used as a model to test the applicability and validity of Hudson's lactone Rule[3] to the (bicyclic) lactone of a γ-hydroxyamino acid. The configuration of VIIa is fixed in an unequivocal manner, since C(2) has the natural $\mathrm{L_S}$-arrangement and since the lactone requires the *cis*-arrangement for the C(2), C(4) substituents involved. The absolute configuration at C(4) is then that of D-glyceraldehyde and leads to the designation $\mathrm{D_G}$. Table I shows that the rotational data and the application of Hudson's Rule[4] agree with the $\mathrm{D_G}$-configuration of C(4) as required by chemical evidence.

The application of Hudson's Rule to the *cis*- and *trans*-disubstituted γ-lactones (IIIb, IVb) of α, δ-dihexahydrobenzoyl-L-*threo*- and L-*erythro*-γ-hydroxyornithine (IIb; Vb) became possible when a suitable solvent for these lactones was found. The solvent used up to now has been pyridine in which both lactones show negative rotations (Table). Pyridine, especially for lactones with small rotatory power, should apparently be avoided when configurational assignments are at stake. Dimethyl sulfoxide, m.p. 18·5°[5], on slight warming, dissolved the lactones IIIb and IVb easily and was miscible with the standardized methanolic KOH solution. The results in the Table show again agreement of configurational assignments by chemical and rotational data.

The ring homolog of natural hydroxy-L-proline (VI) is 5-hydroxy-L-pipecolic acid (XII) occurring naturally in dates and other plants. Its configuration (XII) is exactly analogous to that of hydroxy-L-proline (VI)[6]. The conversion of XII, *via* $NaBH_4$ reduction of the N-carbobenzyloxy-5-keto-L-pipecolic acid to allohydroxy-derivatives (XIVa, XIVb) and lactonization to XIIIb and XIIIa established the configurations at C(2) and C(5) in the same unambiguous way as in the case of VIIb and VIIa. The Table shows again the applicability of Hudson's Rule to this lactone XIIIb.

δ-Hydroxy-L-lysine, despite numerous investigations on its isolation and synthesis[7], is the last important amino acid of unknown configuration. Its lactone is not

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[1] B. Witkop and T. Beiler, J. Amer. chem. Soc. *78*, 2882 (1956).

[2] A. A. Patchett and B. Witkop, J. Amer. chem. Soc. *78*, December 1956.

[3] C. S. Hudson, J. Amer. chem. Soc. *32*, 338 (1910); *61*, 1525 (1939). – E. Anderson, J. Amer. chem. Soc. *34*, 51 (1912). – *cf.* F. Bates and Associates, *Polarimetry, Saccharimetry and the Sugars*, Circular of the National Bureau of Standards C440 (U. S. Government Printing Office, Washington 1942), p. 434.

[4] In its simplest form Hudson's lactone Rule says: γ- or δ-(sugar) lactones in which the carbon carrying the hydroxyl group is related to D-glyceraldehyde are dextrorotatory, or $[\alpha]_D$ (lactone)-$[\alpha]_D$ (acid) is positive. The opening of the lactones with one equivalent of base, as recorded in the Table, leads to the alkali salts of the open acids which in the examples studied were all N-acyl- or N,N'-diacylamino acids. Only minor rotational changes were observed when the solutions, after completion of mutarotation by base, were brought to a pH of 1.

[5] T. B. Douglas, J. Amer. chem. Soc. *68*, 1072 (1946). – The compound was obtained from: The Stepan Chemical Company, 20 North Wacker Drive, Chicago 6, Illinois.

[6] B. Witkop and C. M. Foltz, J. Amer. chem. Soc. *78*, December (1956).

[7] J. C. Sheehan and W. A. Bolhofer, J. Amer. chem. Soc. *72*, 2466, 2472 (1950). – O. Touster, J. Amer. chem. Soc. *73*, 491 (1951). – G. van Zyl, E. E. van Tamelen, and G. D. Zuidema, J. Amer. chem. Soc. *73*, 1765 (1951). – W. S. Fones, J. Amer. chem. Soc. *75*, 4865 (1953). – J. R. Weisiger, J. biol. Chem. *186*, 591 (1950). – S. Bergstrom and S. Lindstedt, Arch. Biochem. *26*, 323 (1950).

Rotations of γ- and δ-lactones of various γ- and δ-hydroxyamino acids and the rotational changes on addition of one equivalent of base.

Compound	M.p.	$[\alpha]^{20}_{D}$ of lactone[1]	$[\alpha]^{20}_{D}$ after addition of one equ. KOH[2]	$\Delta[\alpha]^{20}_{D}$ lactone-open salt or acid	Solvent	Configuration at γ- or δ-Carbon	
						By Hudson's Rule	By Chemical Evid.
trans-disubstituted γ-lactone of α,δ-dihexahydrobenzoyl-L-*erythro*-γ-hydroxyornithine (IVb)	243[3]	− 32·7 − 35·4	− 4·0 + 6·5	− 28·7 − 41·9	dimethyl sulfoxide pyridine	L$_G$ L$_G$	L$_G$
cis-disubstituted γ-lactone of α,δ-dihexahydrobenzoyl-L-*threo*-γ-hydroxyornithine (IIIb)	263[3]	+ 6·4 − 1·5	+ 3·8 + 7·3	+ 2·6 − 8·4[4]	dimethyl sulfoxide pyridine[5]	D$_G$ –	D$_G$
cis-γ-lactone of N-carbobenzyloxy-γ-allohydroxy-L-proline (VIIb)	103	+ 27·2 + 13·2	− 35·7 − 44·3	+ 62·9 + 67·5	dimethyl sulfoxide pyridine	D$_G$ D$_G$	D$_G$
cis-δ-lactone of N-carbobenzyloxy-5-allohydroxy-L-pipecolic acid (XIIIb)	106	− 6·3	− 39·3	+ 33·0	methanol	D$_G$	D$_G$
trans(?)-disubstituted δ-lactone of α,ε-dibenzoyl-δ-hydroxy-L-lysine (XV)	216–218	+ 42·8 + 61·6	− 2·6 − 4·0[6]	+ 45·4 + 65·6	dioxan ethanol[7]	D$_G$ D$_G$	missing

[1] The concentrations of the solutions in this column were kept close to $c = 1{\cdot}0$.

[2] The concentrations of the solutions in this column were kept close to $c = 0{\cdot}5$, the solutions contained 50% CH_3OH.

[3] This sample was an especially pure one obtained by reduction of α,δ-dibenzoyl-γ-keto-L-ornithine methyl ester in methanol with Raney Nickel [B. Witkop and T. Beiler, J. Amer. chem. Soc. *78*, 2882 (1956)].

[4] After 24 h the rotation had changed to $[\alpha]^{20}_{D}$ + 5°; this value fell to 0° on acidification with a few drops of concentrated hydrochloric acid.

[5] A sample from mother liquors of IIIb, m.p. 256–258°, consisting of pure *cis*-lactone according to infrared evidence, but containing some mono- or dibenzoyllactone, was soluble in hot dioxan, $[\alpha]^{20}_{D}$ -11·6° (*c*, 0·9); after addition of one equ. of KOH, $[\alpha]^{20}_{D}$ − 22·7° (*c*, 0·5); $\Delta[\alpha]^{20}_{D}$ + 11·1°, confirming the D$_G$-configuration.

[6] This is the rotationof α, ε-dibenzoyl-D-hydroxylysine (XVIb) as recorded by W. S. Fones, J. Amer. chem. Soc. *75*, 4865 (1953).

[7] The sample used for rotation in ethanol was somewhat purer than that used in dioxan.

known, since δ-lactones are less readily isolated compared with γ-lactones, such as IIIa and IVa. An interesting difference exists between the diacyl derivatives of normal and allohydroxylysine. The two diastereoisomeric α-N-chloroacetyl-ε-N-carbobenzyloxy-δ-hydroxy-D, L-lysines both exist as lactones (m.p. 150–152° and 143–145°)[8], whereas of the two diastereoisomeric α,ε-dibenzoyl derivatives that of δ-hydroxy-D-lysine has been reported as the acid[9], and that of δ-allohydroxy-L-lysine as the lactone (m.p. 196–198°). It was now found that α,ε-dibenzoyl-δ-hydroxy-D-lysine (m.p. 176–178°)[10], as the free acid or its sodium salt, on treatment with excess *p*-tolylsulfonyl chloride in pyridine[11], gave in good yield a neutral compound, recrystallizable from dioxan, m.p. 216–218°. *Anal.* Calcd. for $C_{20}H_{20}N_2O_4$: C, 68·17; H, 5·72; N, 7·95. Found: C, 68·16; H, 5·89; N, 8·15. The Table shows that the application of Hudson's Rule to the rotation of this lactone indicates the D$_G$-configuration for C(5) in δ-hydroxy-D-lysine, which would then be expressed by the structure XVIa and by XVIb and XV for the two dibenzoyl derivatives. If this is correct, natural δ-hydroxy-L-lysine as obtainable from collagen or gelatin[12] is XIa and has the *erythro*-configuration identical with its *trans*-disubstituted cyclization product[13], namely natural 5-hydroxy-L-pipecolic acid (XII), a view which has been expressed before[14]. The final acceptance of this conclusion is awaiting further chemical evidence, especially so, since *a priori* the *trans*-disubstituted δ-lactones X and XV with two equatorial substituents would be expected to form more easily than the *cis*-1,4-disubstituted lactone IX[15] with one equatorial and one axial substituent.

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[8] W. S. Fones, J. Amer. chem. Soc. *75*, 4865 (1953).

[9] J. R. Weisiger, J. biol. Chem. *186*, 591 (1950). – S. Bergstrom and S. Linstedt, Arch. Biochem. *26*, 323 (1950).

[10] I am greatly indebted to Dr. J. R. Weisiger, The Rockefeller Institute of Medical Research, New York, for a sample of this compound.

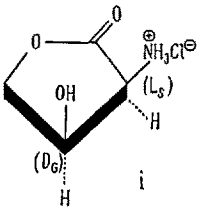
[11] The action of acetic anhydride which converted XIVb smoothly to XIIIb, was without effect.

[12] *Cf.* P. B. Hamilton and R. A. Anderson, J. biol. Chem. *213*, 249 (1955).

[13] *Cf.* L. A. Cohen, F. Irreverre, K. A. Piez, B. Witkop, and H. L. Wolff, Science *123*, 842 (1956).

[14] B. Witkop, Special Publication No. 3 (The Chemical Society, Burlington House, W. 1, London 1955), p. 60.

[15] For a pair of diastereoisomeric β-substituted γ-hydroxyamino acids, the following dualism with respect to lactonization exists: D$_G$-*threo*-α-amino-β,γ-dihydroxybutyric acid forms the *trans*-disubstituted lactone on N-benzoylation, whereas the D$_G$-*erythro*-isomer yields the open benzamido acid under the same conditions. On the other hand, when both D$_G$-*threo*- and *erythro*-α-amino-β,γ-dihydroxybutyric acids are treated with HCl in ethanol, only the *cis*-disubstituted D-*erythro*-lactone is formed, the *threo*-acid does not form a lactone [E. E. Hamel and E. P. Painter, J. Amer. chem. Soc. *75*, 1362 (1953). – J. Kiss, G. Fodor, and D. Bánfi, Helv. chim. Acta *37*, 1471 (1954)]. It should also be noted that surprisingly enough a constant rotation, $[\alpha]_D$ + 51·2°, has been reported (*loc. cit.*) for the



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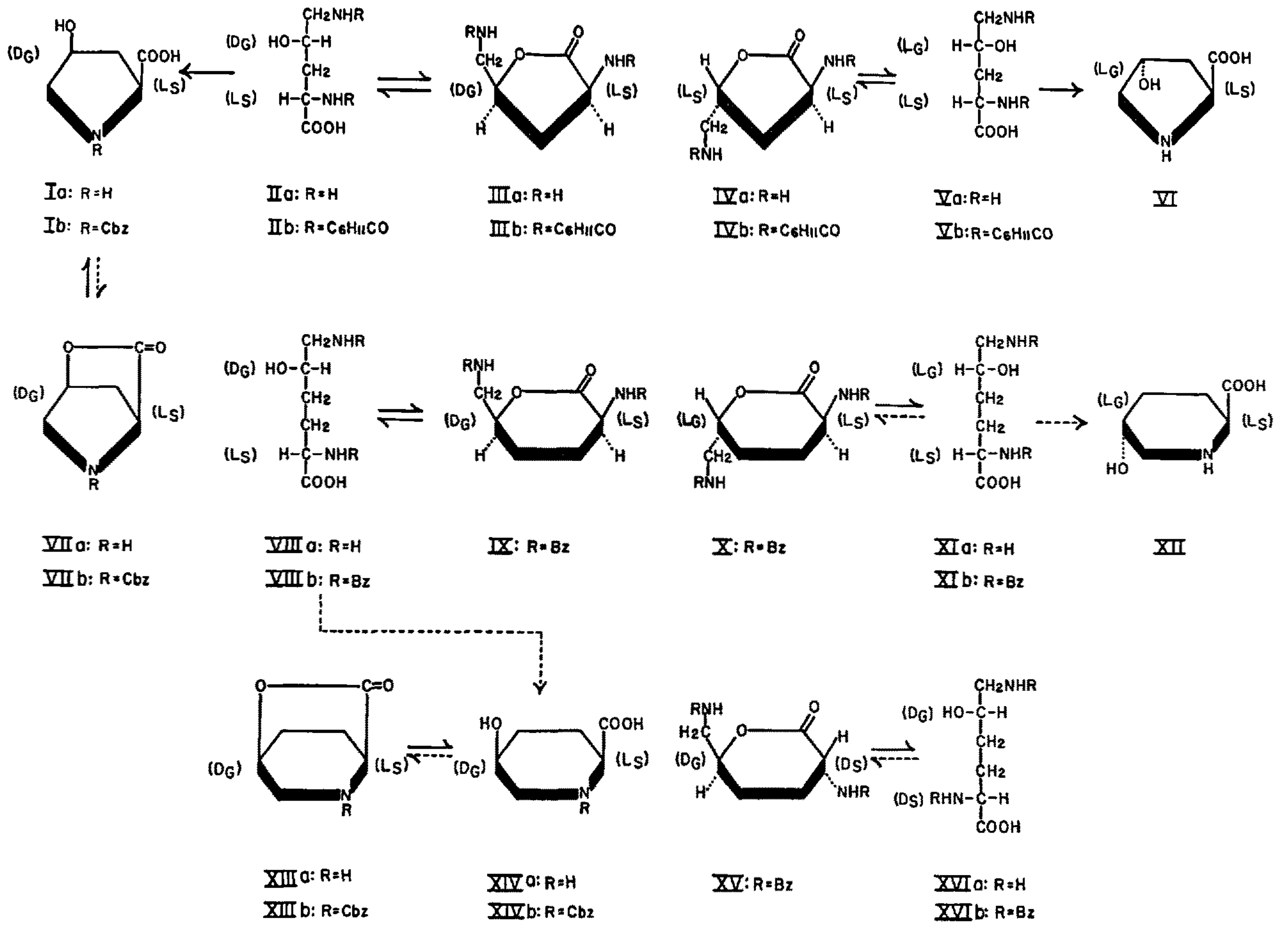

Configurations and correlations of γ- and δ-hydroxyamino acids and their lactones deduced from chemical and, for IX, X, XI, XV and XVI, rotational data. The designations D_G and L_G refer to D- and L-glyceraldehyde and D_S and L_S to D- and L-serine [cf. H. B. VICKERY, J. biol. Chem. *169*, 242 (1947); Chem. Eng. News *25*, 1365 (1947)].

With our increasing understanding of the structure of collagen[16] the confirmation of the absolute configuration of δ-hydroxy-L-lysine is of considerable importance.

It is a pleasure to thank Mrs. EVELYN G. PEAKE for the measurement of the rotations.

B. WITKOP

National Institutes of Health, Washington 14, D. C., July 27, 1956.

Zusammenfassung

Die Anwendung von Hudsons Lakton-Regel auf eine Reihe von Laktonen von γ- und δ-Oxyaminosäuren bestätigte die unabhängig auf chemischem Wege bewiesenen Konfigurationen an den γ- und δ-Kohlenstoffatomen. Im Falle des erstmalig dargestellten Laktons des α, ε-Dibenzoyl-δ-hydroxy-D-lysins führte die Anwendung der Regel zur *erythro*-Struktur XV, und folglich für natürliches δ-Oxylysin aus Collagen zur Formel XIa, deren Beweis auf chemischem Wege noch aussteht.

Electrochemical Behaviour of β-Carotene with Strong Acids

In previous communications we dealt with the electrolytic behaviour of some carotinoids in strongly acid media[1]. We now proceeded to examine whether it was possible to obtain "blue" solutions of β-carotene with other strong acids beside sulfuric acid, which was known up to now, by choosing suitable common solvents for acid and carotene. We succeeded in doing so and were thus able to examine the electrolytic behaviour of these blue solutions as well.

Our apparatus was essentially the one described[2], with the difference that the inverted V-tube was immersed into vessels of an internal diameter of 14 mm together with the Pt electrodes.

Hydrogen chloride in glacial acetic acid slowly turned blue with a drop of carotene in benzene, and the colour migrated towards the cathode. Presence of some water did not alter the result. Its total exclusion with acetic anhydride yielded a blue solution only if air was excluded also. As in all subsequent cases, the colour gradually changed and faded at last after some time: the mobilities and in some cases even the direction of migration were apt to change with this change of colour. We are evident-

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lactone hydrochloride (i), whereas the hydrobromides of the lactones IIIa, IVa, VIIa and XIIIa show rapid mutarotation with the rate of ring-opening increasing qualitatively in the order IIIa $\cong$ IVa < VIIa < XIIIa.

[16] A. RICH and F. H. C. CRICK, Nature *176*, 915 (1955).

[1] F. KÖRÖSY, Exper. *11*, 342 (1955); Magy. kém. Foly. *61*, 155 (1955).

[2] F. KÖRÖSY and G. SZÉKELY, Magy. kém. Foly. *58*, 174 (1952).