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Reductive Cyclization. A Method for the Synthesis of Tricyclic Compounds Possessing a Bridgehead Nitrogen[1]

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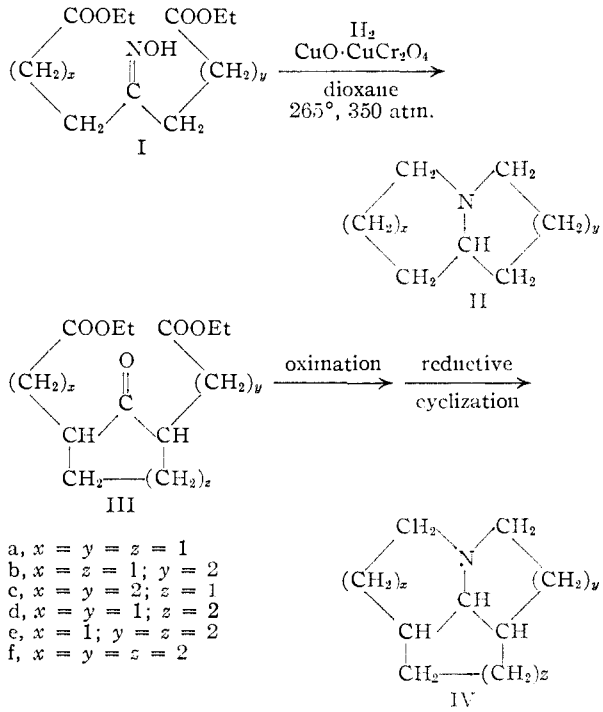
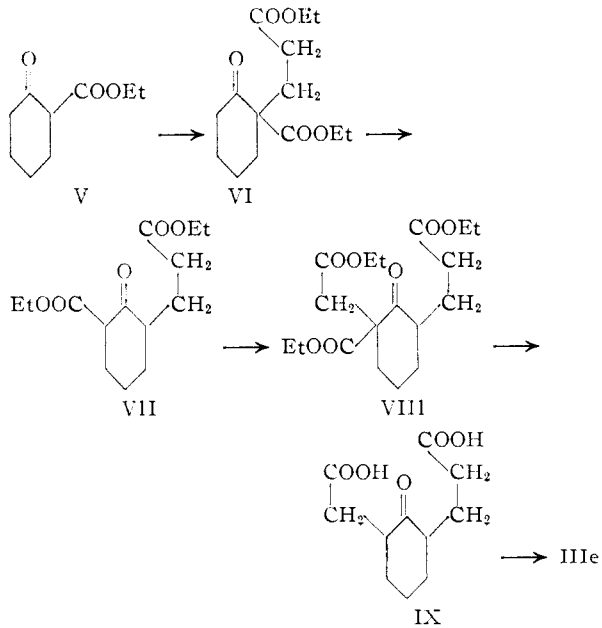
The general method of reductive cyclization of oximinodiesters—by hydrogenation over copper chromite at high temperature and pressure—has been extended to the synthesis of tricyclic compounds with a bridgehead nitrogen. The oximino diesters used as precursors were monocyclic, possessing a five- or six-membered ring, so that with the formation of two new five- or six-membered rings in the reductive cyclization process, the method affords a variety of tricyclic tertiary amines. Hexahydrojulolidine, obtained by the reductive cyclization of the oxime of diethyl cyclohexanone-2,6-di-β-propionate, served as a model for the general synthetic method since it was a known compound. Hexahydrolilolidine was obtained similarly by the reductive cyclization of the oxime of diethyl cyclohexanone-2-acetate-6-β-propionate. 1-Azatricyclo[6.3.1.0^{5,12}]-dodecane and 1-azatricyclo[6.2.1.0^{5,11}]hendecane were obtained from the analogously-substituted cyclopentanone oximes. It has been possible to assign stereochemical configurations to the tricyclic bases thus obtained by consideration of the following: (a) the probable stereochemical structure of the precursor keto diesters; (b) a comparison of the scale molecular models for the isomeric fully-reduced bases; (c) chromatography on D-lactose hydrate, employed as a qualitative tool for distinguishing between *racemic* and *meso* forms where these are possibilities.

The reductive cyclization of oximino diesters (I) over copper chromite catalyst at high temperature and high hydrogen pressure has been shown to be a general method for the synthesis of 1-azabicyclo compounds (II).[2] A logical extension of this synthetic method would be its application to the preparation of 1-azatricyclo compounds (IV) from the oximes of α,α′-di-(carbethoxyalkyl)-cycloalkanones of type III. Six esters of this type (IIIa–f) were prepared and four of these (b, c, e, f)



were successfully converted, by oximation and subsequent reductive cyclization, to the corresponding 1-azatricyclo compounds.

The keto diesters (III) were prepared by the general method discovered by Openshaw and Robinson[3] for cyclohexanone derivatives and applied by Chaterjee and his co-workers[4,5] to cyclopentanone derivatives. The method can be illustrated by the specific case of diethyl cyclohexanone-2-acetate-6-β-propionate (IIIe). Ethyl cyclohexanone-2-carboxylate (V), as the sodio derivative, was treated with ethyl β-bromopropionate to give diethyl cyclohexanone-2-carboxylate-2-β-propionate (VI), which was isomerized to diethyl cyclohexanone-2-carboxylate-6-β-propionate (VII) by refluxing in ethanolic sodium ethoxide.[3] Compound VII, as the sodio derivative, was treated



with ethyl bromoacetate to give triethyl cyclohexanone - 2 - acetate - 2 - carboxylate - 6 - β - propionate (VIII), which was converted, by acid hydrolysis (to IX) and subsequent esterification, to IIIe. Many of the esters listed in Table I have been prepared previously,[3,4,5] but since they were insufficiently characterized as to refractive index and density (and molecular refractivity), their physical properties are given in some detail.

(1) This work was supported by a grant from E. I. du Pont de Nemours and Company, Inc.

(2) N. J. Leonard and W. E. Goode, THIS JOURNAL, **72**, 5404 (1950).

(3) H. T. Openshaw and R. Robinson, *J. Chem. Soc.*, 941 (1937).

(4) N. N. Chaterjee, B. K. Das and G. N. Barpujari, *J. Indian Chem. Soc.*, **17**, 161 (1940).

(5) N. N. Chaterjee and A. Bose, *Science and Culture*, **6**, 724 (1941).

TABLE I

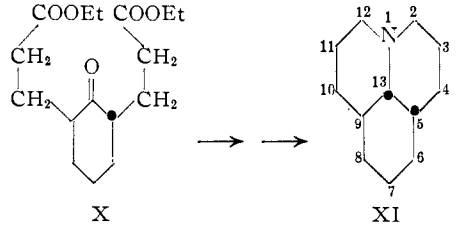
KETO ESTERS

Group	Compound	Yield, %	B.p. °C.	Mm.	$n^{20}D$	d^{20}_4	MRD Calcd.	MRD Found
A	Diethyl cyclopentanone-2-acetate-2-carboxylate[4]	83	110–112	0.5	1.4559			
	Diethyl cyclopentanone-2-carboxylate-2-β-propionate[4]	75	187–190	18	1.4591			
	Diethyl cyclohexanone-2-acetate-2-carboxylate[5]	72	130–132	1.0	1.4613			
	Diethyl cyclohexanone-2-carboxylate-2-β-propionate[3]	65	150–152	1.0	1.4633			
B	Diethyl cyclopentanone-5-acetate-2-carboxylate[4]	78	148–150	1.1	1.4567	1.1150	58.74	59.14
	Diethyl cyclopentanone-2-carboxylate-5-β-propionate[4]	90	150–153	1.0	1.4558	1.0926	63.35	63.74
	Diethyl cyclohexanone-6-acetate-2-carboxylate[a]	60	165–167	3.0	1.4656	1.1121	63.35	63.78
	Diethyl cyclohexanone-2-carboxylate-6-β-propionate[3]	77	165–170	2.0	1.4490	1.0646	67.97	68.22
C	Triethyl cyclopentanone-2-carboxylate-2,5-diacetate[4]	50	162–167	0.5	1.4601	1.1386	78.86	79.00
	Triethyl cyclopentanone-2-acetate-2-carboxylate-5-β-propionate[4]	56	185–186	1.3	1.4594	1.1194	83.48	83.68
	Triethyl cyclopentanone-2-carboxylate-2,5-di-β-propionate[4]	60	196–198	1.5	1.4632	1.1133	88.10	88.21
	Triethyl cyclohexanone-2-carboxylate-2,6-diacetate[5]	64	193–198	2.8	1.4595	1.1200	83.48	83.65
	Triethyl cyclohexanone-2-acetate-2-carboxylate-6-β-propionate[b]	58	190–192	1.1	1.4744	1.1365	88.10	88.20
	Triethyl cyclohexanone-2-carboxylate-2,6-di-β-propionate[3]	61	213–214	2.8	1.4700	1.1180	92.72	92.70
D[c]	Diethyl cyclopentanone-2,5-diacetate[4]	75	135–137	0.4	1.4628	1.1113	63.35	63.49
	Diethyl cyclopentanone-2-acetate-5-β-propionate[4]	81	163–165	.8	1.4608	1.0905	67.97	68.00
	Diethyl cyclopentanone-2,5-di-β-propionate[4]	91	161–162	.4	1.4633	1.0831	72.59	72.68
	Diethyl cyclohexanone-2,6-diacetate[d]	84	160–165	.7	1.4809	1.1215	67.97	68.59
	Diethyl cyclohexanone-2-acetate-6-β-propionate[e]	92	184–190	3.5	1.4697	1.0905	72.59	72.71
	Diethyl cyclohexanone-2,6-di-β-propionate[3]	80	189–192	1.0	1.4670	1.0840	77.21	77.09

[a] *Anal.* Calcd. for $C_{13}H_{20}O_5$: C, 60.92; H, 7.87. Found: C, 60.82; H, 7.81. [b] *Anal.* Calcd. for $C_{18}H_{28}O_7$: C, 60.66 H, 7.92. Found: C, 60.50; H, 7.90. [c] These keto diesters were obtained by esterification of the corresponding keto diacids obtained by hydrochloric acid hydrolysis of the keto triesters (group C): cyclopentanone-2,5-diacetic acid,[4] 75% yield, m.p. 177°; cyclopentanone-2-acetic-5-β-propionic acid, 80%, m.p. 126–127°; cyclopentanone-2,5-di-β-propionic acid,[4] 90%, m.p. 122°; cyclohexanone-2,6-diacetic acid,[5] 60%, m.p. 188°; cyclohexanone-2-acetic-6-β-propionic acid, 91%, m.p. 167–168° (*Anal.* Calcd. for $C_{11}H_{16}O_5$: C, 57.88; H, 7.07. Found: C, 58.07; H, 7.16); cyclohexanone-2,6,β-propionic acid,[3] 95%, m.p. 145°. [d] *Anal.* Calcd. for $C_{14}H_{22}O_5$: C, 62.20; H, 8.21. Found: C, 61.98; H, 8.13. [e] *Anal.* Calcd. for $C_{15}H_{24}O_5$: C, 63.36; H, 8.51. Found: C, 63.24; H, 8.48.

The method of reductive cyclization of the oximes of the keto diesters of type III was first tested with diethyl cyclohexanone-2,6-di-β-propionate (IIIf), since this precursor should yield hexahydrojulolidine (trivial name),[6] or 1-azatricyclo[7.3.1.$0^{5,13}$]-tridecane (IVf) (systematic name).[7] Hexahydrojulolidine has been obtained previously by the hydrogenation of julolidine[8] in ethanol over Raney nickel at 200° and 14 atmospheres[9,10] and in acetic acid containing hydrobromic acid over platinum oxide at 60° and atmospheric pressure.[10] Two of the three possible isomeric forms of hexahydrojulolidine (*cis–cis*, *cis–trans* and *trans–trans*) were isolated by Protiva and Prelog[10] from the low-pressure reduction of julolidine, and these were designated as isomers A (picrate, m.p. 186–187°; picrolonate, m.p. 265–266°, with decomposition) and B (picrate, m.p. 223–224°). The oxime of diethyl cyclohexanone-2,6-di-β-propionate (IIIf) was prepared and the crude oxime was subjected directly to hydrogenation in dioxane solution over copper chromite at 265° and 350 atmospheres. The tertiary amine which was isolated from the reductive cyclization process (38% yield) had physical properties practically identical with those described for isomer A of hexahydrojulolidine,[10] and formed a picrate, m.p. 185–186°, and picrolonate, m.p. 264–265° (dec.), which supported this identification. A repetition of the hydrogenation and separation procedure of Protiva and Prelog[10] furnished a sample of hexahydrojulolidine-A picrate which was indeed identical with the picrate of IVf prepared by our reductive cyclization process.

It would be most desirable to assign the correct stereochemical configuration to the hexahydrojulolidine isomer thus obtained. An approach in this direction can be made by a consideration of the probable configuration of the precursor keto diester IIIf. It seems very likely that this is predominantly the *trans*-diester (X) rather than the *cis*-diester, since it is given every opportunity to equilibrate in the direction of the more stable (*trans*) form[11]—at the alkoxide stage in its prepara-



X XI

(6) The trivial name is derived from the name "julolidine" given to the analogous compound with a benzene ring in place of the cyclohexane ring (G. Pinkus, *Ber.*, **25**, 2802 (1892)).

(7) The systematic name is derived according to the nomenclature rules set forth in *C. A.*, **39**, 5885 (1945); see also A. M. Patterson and L. T. Capell, "The Ring Index," Reinhold Publishing Corporation, New York, N. Y., 1940, p. 26.

(8) *Org. Syntheses*, **26**, 40 (1946).

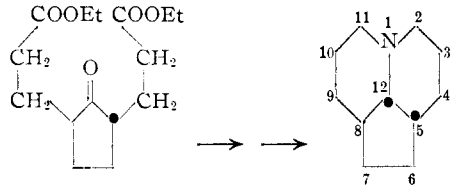
(9) V. Boekelheide and G. P. Quinn, THIS JOURNAL, **70**, 2830 (1948).

(10) M. Protiva and V. Prelog, *Helv. Chim. Acta*, **32**, 621 (1949).

(11) T. L. Jacobs, R. Reed and E. Pacovska, THIS JOURNAL, **73**, 4505 (1951); see also D. H. R. Barton, *Experientia*, **6**, 312 (1950).

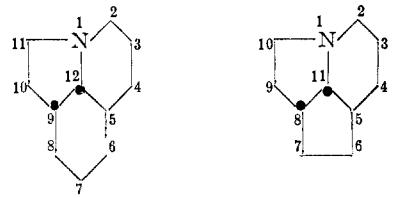
tio n(corresponding to VII → VIII with the lower homolog) and during the hydrolysis and reesterification stages, both in acidic media. If the diethyl cyclohexanone-*trans*-2,6-di-β-propionate (X, one enantiomorph is shown) is the precursor of IVf, this relative configuration would not be expected to be altered in the reductive cyclization process, so that the hexahydrojulolidine obtained should be the *cis–trans* isomer (XI, one enantiomorph is shown: the C_{13}-hydrogen *cis*, and the C_9-hydrogen *trans*, to the C_5-hydrogen). The *cis–trans* form of hexahydrojulolidine is racemic, whereas the *cis–cis* and *trans–trans* forms are *meso* forms. Hence, if isomer A were resolvable, it would necessarily have the *cis–trans* configuration (X). Since complete resolution is usually a tedious process, a quicker, qualitative method was sought for confirming the structure (X) hypothesized for hexahydrojulolidine-A. A solution of pure hexahydrojulolidine-A in petroleum ether was chromatographed on a column of pure D-lactose hydrate,[12] and the chromatogram was developed with additional petroleum ether. The percolate was discarded until picrate formation and basicity to moist litmus paper indicated the presence of amine. A basic percolate was then collected, which, upon concentration, was found to give a measurable *positive* rotation. The succeeding basic percolate collected from the column had a demonstrable *negative* rotation after concentration. This behavior is indicative of the preferential adsorption of the (−) form of hexahydrojulolidine-A on the column, and, more important, shows that the base chromatographed can exist in optically active forms. Since only the *cis–trans* isomer of hexahydrojulolidine (XI) has this possibility, structure XI can be assigned definitely to the $C_{12}H_{21}N$ product of reductive cyclization of IIIf (*viz.*, X). This structure can be rationalized as being the product also of the catalytic hydrogenation of julolidine[9,10] on the basis of the *cis* addition of pairs of adjacent hydrogen atoms to the double-bond system of the benzenoid ring. This concept would also lead one to predict that the additional hexahydrojulolidine isomer (B)[10] produced by the catalytic hydrogenation of julolidine is the *meso* form of IVf having the *cis–cis* configuration. A sample of hexahydrojulolidine-B, carefully purified through the picrate,[10] was chromatographed on a D-lactose hydrate column. No optical activity could be detected in any basic percolate fraction.[13]

The method of chromatographic adsorption and partial development also permitted assignment of stereochemical configuration to the isomer of 1-azatricyclo[6.3.1.$0^{5,12}$]dodecane (IVc), $C_{11}H_{19}N$ (picrate, m.p. 211–212°; picrolonate, m.p. 232–233°) obtained by the reductive cyclization of the oxime of diethyl cyclopentanone-2,5-di-β-propionate (IIIc). The homogeneous reduction product was chromatographed on D-lactose hydrate, and the first portion of percolate which contained amine was measurably dextrorotatory. This isomer of 1-azatricyclo[6.3.1.$0^{5,12}$]dodecane therefore had to be the racemic (*cis–trans*) form (XIII) rather than either of the *meso* forms (*cis–cis* or *trans–trans*). The assignment of the *cis–trans* structure (XIII and its mirror image) further establishes the configuration of the precursor keto diester as *trans* (XII), which is consistent with the probable formation of the more stable (*trans*)[14] isomer of diethyl cyclopentanone-2,5-di-β-propionate by its mode of synthesis.



XII XIII

The chromatography method is not applicable to the problem of deciding which isomer of hexahydrolilolidine (IVe),[15] or 1-azatricyclo[7.2.1.$0^{5,12}$]-dodecane, was obtained by the reductive cyclization of the oxime of diethyl cyclohexanone-2-acetate-6-β-propionate (IIIe), since all of the four possible forms of hexahydrolilolidine (*cis–cis*, *trans–cis*, *cis–trans* and *trans–trans*) are racemic. On the basis of the arguments for structure assignments X and XII, however, it seems reasonable that the precursor keto diester IIIe would have the *trans* configuration.[11] Accordingly, the $C_{11}H_{19}N$ product of catalytic hydrogenation would be limited to the *trans–cis* (XIV) or *cis–trans* (C_{12}–H *cis*, and C_9–H *trans*, to C_5–H) configuration, and



XIV XV

assignment of stereochemical structure is *tentatively* made as XIV (racemate), since the *trans–cis* form is the more readily constructed in scale molecular models. Following similar arguments (*trans* structure for the precursor ester IIIb,[14] easier construction of the scale molecular models for the *trans–cis* as compared with the *cis–trans* form of IVb), the stereochemical structure XV (racemate) is *tentatively* assigned to the isomer of 1-azatricyclo[6.2.1.$0^{5,11}$]hendecane (IVb) which resulted from the reductive cyclization of the oxime of diethyl cyclopentanone-2-acetate-5-β-propionate (IIIb). The properties of the 1-azatricyclo products are given in Table II.

It is instructive that the reductive cyclization process, when attempted with the oxime of diethyl cyclopentanone-2,5-diacetate (IIIa), did not pro-

(12) V. Prelog and P. Wieland (*Helv. Chim. Acta*, **27**, 1127 (1944)) resolved Tröger's base by chromatography on D-lactose hydrate.

(13) Such negative evidence for structure assignment is not as conclusive as the successful qualitative resolution which established the structure of isomer A. For example, octahydropyrrocoline, which is a racemate, gave no optically active percolate fractions when chromatographed on D-lactose hydrate.

(14) T. L. Jacobs and W. R. Florsheim, THIS JOURNAL, **72**, 256, 261 (1950).

(15) The trivial name is derived from the name "lilolidine" given to the analogous compound with a benzene ring in place of the cyclohexane ring (E. Bamberger and H. Sternitzki, *Ber.*, **26**, 1291 (1893)).

TABLE II

AMINES

Amine	Yield,[a] %	B.p. °C.	Mm.	$n^{20}D$	d^{20}_4	Formula	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found	MR_D Calcd.	Found
1-Azatricyclo[6.2.1.$0^{5,11}$]hendecane	19	45	0.2	1.4982	0.9657	$C_{10}H_{17}N$	79.34	79.39	11.33	11.23	9.33	9.02	45.72	45.92
1-Azatricyclo [6.3.1.$0^{5,12}$] dodecane	26	56	.5	1.5037	.9671	$C_{11}H_{19}N$	79.94	79.64	11.59	11.54	8.47	8.17	50.34	50.58
1-Azatricyclo [7.2.1.$0^{5,12}$] dodecane (hexahydrolilolidine)	25	56	.8	1.4988	.9606	$C_{11}H_{19}N$	79.94	79.84	11.59	11.40	8.47	8.27	50.34	50.50
1-Azatricyclo [7.3.1.$0^{5,13}$] tridecane (hexahydrojulolidine, isomer A)[10]	38	80	.8	1.5034	.9601	$C_{12}H_{21}N$	80.38	80.23	11.81	11.59	7.81	7.70	54.96	55.24

DERIVATIVES OF AMINES

Amine	Picrate M.p., °C.	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Picrolonate M.p., °C.	Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found
1-Azatricyclo[6.2.1.$0^{5,11}$]hendecane	242–243 dec.	50.52	50.79	5.30	5.29	183–184	57.82	58.01	6.07	6.16
1-Azatricyclo [6.3.1.$0^{5,12}$] dodecane	211–212	51.77	52.05	5.62	5.86	232–233	58.73	58.53	6.34	6.31
1-Azatricyclo [7.2.1.$0^{5,12}$] dodecane (hexahydrolilolidine)	210–211	51.77	51.61	5.62	5.60	205–206 dec.	58.73	58.63	6.34	6.30
1-Azatricyclo [7.3.1.$0^{5,13}$] tridecane (hexahydrojulolidine, isomer A)[10]	185–185	52.93	52.94	5.92	6.03	264–265 dec.	59.58	59.43	6.59	6.60

[a] Yield of 1-azatricyclo compound based on the keto diester. The 1-azatricyclo compounds were homogeneous. Higher-boiling products were not isolated and characterized.

duce any 1-azatricyclo[5.2.1.$0^{4,10}$]decane (IVa). Scale molecular models predict the difficulty of formation of the *cis–trans* isomer with this ring system. We also failed to isolate any 1-azatricyclo[6.2.1.$0^{4,11}$]hendecane (IVd) from the oxime of diethyl cyclohexanone-2,6-diacetate (IIId) in an attempted reductive cyclization.

Experimental[16]

1-Azatricyclo Compounds.—The general method for the preparation and reductive cyclization of oximino diesters to 1-azatricyclo compounds is illustrated below in the directions for the preparation of hexahydrojulolidine.

Hexahydrojulolidine.—A few drops of glacial acetic acid was added to a solution of 14.9 g. (0.05 mole) of diethyl cyclohexanone-2,6-di-β-propionate, 9.8 g. (0.1 mole) of potassium acetate and 6.95 g. (0.1 mole) of hydroxylamine hydrochloride in 100 ml. of 50% ethanol. The solution was heated under reflux for 3 hours, cooled, diluted with water and extracted with chloroform. The chloroform extracts were dried, and the chloroform was removed. The sirupy residue which remained was the crude oxime, which was dissolved in 100 ml. of purified dioxane and reduced with hydrogen in the presence of 10 g. of copper chromite catalyst at 265° and 350 atmospheres. After 4 hours, the theoretical amount of hydrogen was absorbed. The catalyst was removed by filtration and the filtrate was fractionated at reduced pressure. Hexahydrojulolidine boiled at 80° (0.8 mm.) (see Table II for properties).

Activation of D-Lactose Hydrate.[12]—Two kilograms of commercial crystalline D-lactose hydrate was boiled for 5 minutes with 1.5 l. of freshly distilled chloroform. The hot suspension was filtered and washed repeatedly with hot chloroform. The lactose was dried 1 hour in air and 30 hours in vacuum. The lactose was then ground in a sillimanite ball mill for 40 hours, passed through a No. 45 sieve (324 meshes/sq. cm.), and dried in vacuum for 50 hours before use.

Partial Resolution of 1-Azatricyclo Compounds.—Careful fractional distillation of the 1-azatricyclo[6.3.1.$0^{5,12}$]dodecane and the hexahydrojulolidine prepared by reductive cyclization, and fractional crystallization of their picrates failed to reveal the presence of more than one isomer in each case. Both bases were purified by redistillation shortly before their chromatographic treatments.

Partial Resolution of Racemic 1-Azatricyclo[6.3.1.$0^{5,12}$]-dodecane.—A glass chromatographic cylinder (3.7 × 50 cm.) was packed with activated D-lactose hydrate to a depth of 40 cm. (about 315 g.), and the column was wet with anhydrous petroleum ether (b.p. 45–55°). A solution of 0.7 g. of 1-azatricyclo[6.3.1.$0^{5,12}$]dodecane in 25 ml. of petroleum ether was added to the column, and the chromatogram was developed with additional quantities of petroleum ether. The pressure at the top of the column was adjusted so that a flow of about 100 ml. per hour was obtained. Polarimeter readings were taken every 10 ml. after the initial 235-ml. portion was rejected because it contained no base. A maximum polarimeter reading, $a = 0.08 \pm 0.01°$ (l 2 dm.), was obtained by concentration of the first 80 ml. of basic fraction to 10 ml.

Hexahydrojulolidine Picrates (Isomers A and B).—Thirty-six grams of hexahydrojulolidine (mixture of isomers) prepared by the reduction of julolidine[8] in hydrobromic acid with hydrogen in the presence of platinum oxide, according to the method of Protiva and Prelog,[10] was converted into 57.0 g. of the picrate derivative. By systematic fractional crystallization from ethanol, the picrate was separated into two isomers. Eighteen grams of the less soluble isomer, designated as isomer A picrate,[10] m.p. 185–186°, and 3.25 g. of the more soluble isomer, designated as isomer B picrate,[10] m.p. 222–223°, were obtained in pure form. The first picrate (A) was identical, as established by mixed melting point and infrared comparison, with the picrate obtained *via* our reduction of the oximino diester (see above).

Hexahydrojulolidine, Isomer A.[10]—To a slurry of 18.0 g. of hexahydrojulolidine picrate, isomer A, in 100 ml. of water, was added 90 ml. of concentrated hydrochloric acid, and the mixture was shaken for 30 minutes. The picric acid was removed by filtration, and the filtrate was cooled and made basic with cold 40% potassium hydroxide solution. The alkaline solution was extracted with ether and the extracts were dried over anhydrous sodium sulfate. The ether was evaporated and the residue was distilled under reduced pressure, yielding 7.0 g. (89%) of hexahydrojulolidine, isomer A, b.p. 70° (0.4 mm.); $n^{20}D$ 1.5036; d^{20}_4 0.9605 (constants practically identical with those given in Table II).

Hexahydrojulolidine, Isomer B.—To a slurry of 3.0 g. of hexahydrojulolidine picrate, isomer B, in 15 ml. of water, was added 15 ml. of concentrated hydrochloric acid, and the mixture was shaken for 30 minutes. The picric acid was removed by filtration, and the filtrate was cooled and made basic with cold 40% potassium hydroxide solution. The alkaline solution was extracted with ether, and the ether extracts were dried over anhydrous sodium sulfate. The ether was evaporated and the residue distilled under reduced pressure, yielding 1.03 g. of hexahydrojulolidine, isomer B; b.p. 75° (0.4 mm.); $n^{20}D$ 1.5132.

Hexahydrojulolidine Picrolonate, Isomer B.—Prepared in ether and recrystallized from ethanol, the picrolonate formed ochre prisms which melted at 245–246° with decomposition.

Anal. Calcd. for $C_{22}H_{29}N_5O_5$: C, 59.58; H, 6.59. Found: C, 59.85; H, 6.49.

Partial Resolution of Hexahydrojulolidine. Isomer A.—A glass chromatographic cylinder (7.5 × 50 cm.) was packed with activated D-lactose hydrate to a depth of 45 cm. (about 1450 g.) and the column was wet with dry petroleum ether (b.p. 45–55°). A solution of 3.0 g. of hexahydrojulolidine, isomer A (isolated from the reduction of julolidine) in 25 ml. of petroleum ether was added to the column and the chromatogram was developed with additional quantities of petroleum ether. The pressure at the top of the column

(16) All melting points are corrected.

was adjusted so that a flow of about 300 ml. per hour was obtained. The percolate (850 ml.) was discarded until the presence of base was indicated by picrate formation and basicity to moist litmus paper. The first 100 ml. of basic fraction was collected, concentrated to 10 ml., and the optical activity was determined. This process was repeated for the next six 100-ml. fractions. The first four basic fractions showed a slight positive rotation, while the remainder of the fractions had such small rotations that they could not be determined. A maximum positive polarimeter reading, $a = 0.13 \pm 0.01°$ (l 2 dm.), was obtained by combining and concentrating the first four basic fractions to 5 ml. The column was flushed with petroleum ether until the percolate contained no base. This required about 7 liters. All fractions of the basic percolate which did not demonstrate a positive rotation were combined, concentrated to 100 ml., filtered, and further concentrated to 5 ml. A polarimeter reading of $a = -0.06 \pm 0.01°$ (l 2 dm.) was determined for this concentrate.

Blank Determination.—A glass chromatographic cylinder (7.5 × 50 cm.) was packed with activated D-lactose hydrate to a depth of 40 cm. (about 1300 g.) and the column was wet with dry petroleum ether (b.p. 45–55°). Petroleum ether was added to the column, and the pressure at the top of the column was adjusted so that a flow of about 250 ml. per hour was obtained. Two liters of percolate was collected, concentrated to 5 ml., and a polarimeter reading of 0.00 ± 0.01° was determined. A Molisch test with α-naphthol and sulfuric acid failed to reveal the presence of carbohydrate in the percolate. Dry petroleum ether which had been shaken 24 hours with activated D-lactose hydrate and then filtered also failed to give a measurable optical rotation or a positive Molisch test.

Isomer B.—A glass chromatographic cylinder (3.7 × 60 cm.) was packed with activated D-lactose hydrate to a depth of 55 cm. (about 450 g.), and the column was wet with dry petroleum ether (b.p. 45–55°). A solution of 1.0 g. of hexahydrojulolidine, isomer B (from the reduction of julolidine and purification through the picrate) in 20 ml. of petroleum ether was added to the column, and the chromatogram was developed with additional quantities of petroleum ether. The pressure at the top of the column was adjusted so that a flow of about 100 ml. per hour was obtained. The percolate (325 ml.) was discarded until the presence of base was indicated by picrate formation and basicity to moist litmus paper. The first 25 ml. of basic fraction was collected, concentrated to 5 ml., and the optical activity was determined. The next 25-ml. portion was collected, added to the first, and the whole concentrated to 5 ml. and the optical activity again determined. The process of collection, combination, and concentration of the whole was continued until 1000 ml. of basic percolate had been collected and concentrated to 5 ml. After each concentration, the optical activity was determined. In each case, the polarimeter reading was $a = 0.00 \pm 0.01°$ (l 2 dm.).

Attempted Resolution of Octahydropyrrocoline.—A glass chromatographic cylinder (7.5 × 50 cm.) was packed with activated D-lactose hydrate to a depth of 45 cm. (about 1450 g.) and the column was wet with dry petroleum ether (b.p. 45–55°). A solution of 3.0 g. of octahydropyrrocoline[17] in 25 ml. of petroleum ether was added to the column and the chromatogram was developed with additional quantities of petroleum ether. The pressure at the top of the column was adjusted so that a flow of about 300 ml. per hour was obtained. The percolate (800 ml.) was discarded until the presence of base was indicated by picrate formation and basicity to moist litmus paper. The first 25 ml. of basic fraction was collected, concentrated to 5 ml., and the optical activity was determined. The next 50-ml. portion was collected, added to the first, and the whole concentrated to 5 ml. and the optical activity again determined. The process of collection, combination, and concentration of the whole was continued until 2 l. of basic percolate had been collected and concentrated to 5 ml. After each concentration, the optical activity was determined. In each case, the polarimeter reading was $a = 0.00 \pm 0.01°$ (l 2 dm.).

(17) Octahydropyrrocoline was prepared in the manner described by V. Boekelheide and S. Rothchild, THIS JOURNAL, **70**, 864 (1948).

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Sequence of Four Amino Acids at the Amino End of the Single Polypeptide Chain of Lysozyme

BY W. A. SCHROEDER

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Under certain conditions of partial hydrolysis, DNP-lysozyme produces a DNP-tetrapeptide and shorter DNP-peptides but longer peptides have not been isolated. The determination of the structure of the tetrapeptide as α,ε-di-DNP-lysyl-valylphenylalanylglycine thus fixes the sequence of the four amino acids at the amino end of the chain. On the basis of the results, it may be concluded that lysozyme consists of a single polypeptide chain which at the amino end terminates in the above sequence. A quantitative study of the composition of the mixture of end DNP-peptides which is produced by partial hydrolysis indicated the relative ease with which the various peptide bonds may be hydrolyzed: the valyl–phenylalanyl bond is stronger than the lysyl–valyl bond which in turn is stronger than the phenylalanyl–glycyl bond.

Sanger[1] in 1945 first described the use of 2,4-dinitrofluorobenzene (DNFB) for the determination of the free amino groups in proteins and at that time identified the free amino groups of insulin. Further application of the method by Porter and Sanger[2–4] has resulted in the identification of the terminal amino acids at the amino ends of the polypeptide chains of several proteins. Extension of the method by Sanger[5] lead to the isolation of dinitrophenyl-(DNP)-peptides from partial hydrolysates of two fractions of oxidized insulin and the determination of the sequence of the amino acids in these peptides. More recently, the entire sequence of amino acids in one of these fractions has been determined.[6]

In these laboratories, the application of Sanger's method to lysozyme showed that the terminal amino acid of this protein is lysine[7] but it was also found that a DNP-peptide (or peptides) could be isolated from certain hydrolysates. The fact that this DNP-peptide(s) yielded α,ε-di-DNP-lysine on complete hydrolysis lead to the conclusion that it is derived from the amino end of the lysozyme molecule. The present study was designed to

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(2) R. R. Porter and F. Sanger, *ibid.*, **42**, 287 (1948).
(3) R. R. Porter, *Biochim. Biophys. Acta*, **2**, 105 (1948).
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(5) F. Sanger, *Biochem. J.*, **45**, 563 (1949).
(6) F. Sanger and H. Tuppy, *ibid.*, **49**, 463, 481 (1951).
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