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Isolation of Thymidine by Means of the Chromatopile

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Methods have been described recently for the isolation of the desoxyribosides by means of alumina, cation exchange and anion exchange columns.[2-5] The convenient isolation of thymidine from an enzymatic hydrolysate of commercial desoxyribonucleic acid by butanol extraction followed by separation on a chromatopile[6] is reported below.

Sperm nucleic acid[7] (40 g.) was incubated with phosphatase from 240 ml. of calf intestinal mucosa glycerol extract by the method of Klein[8] for 16 hours. The weighed inorganic precipitate, removed by filtration, indicated about 50% hydrolysis (*cf.* Brown and Lythgoe[4]). The solution (2000 ml.) was exhaustively extracted with butanol saturated with water.[9] The remaining aqueous phase contained no ribosides, as determined by paper chromatography. The combined butanol extract was evaporated *in vacuo* to 200 ml., cooled overnight and filtered. The filtrate was evaporated to 35 ml. *in vacuo* and absorbed on double sheets of Whatman #1 filter paper, 12.5 cm. in diameter. After partially drying in air (3.5 hr.) the sheets were incorporated into a 500-sheet pile and developed for 35 hr. at 0° with 1-propanol:0.1 *N* H_2SO_4 (3:1). The thymidine fraction, located in sheets 250 to 375, was eluted with water, neutralized with hot saturated $Ba(OH)_2$ solution to *p*H 6.5 and evaporated *in vacuo* to a small volume. No crystals appeared on standing for three months. When seeded[10] the solution set to a crystalline mass within 30 seconds. The slightly wet crystalline precipitate (1.5 g.) was recrystallized twice from water, washed with ethanol and dried over P_2O_5; yield 0.9 g., m.p. 185–186°. The relative spectra agreed within experimental error with those reported by Hotchkiss,[11] $\lambda_{max.}$ (0.1 *N* HCl) 267 mμ, log ε 4.021, $\lambda_{min.}$ 235 mμ, log ε 3.355, $\lambda_{max.}$ (0.1 *N* NaOH) 266 mμ, log ε 3.942, $\lambda_{min.}$ 246 mμ, log ε 3.751, N_{260}[12] is 38.5.

(1) University of California School of Medicine, Los Angeles 24, Calif.

(2) O. Schindler, *Helv. Chim. Acta*, **32**, 979 (1949).

(3) P. Reichard and B. Estborn, *Acta Scand. Chem.*, **4**, 1047 (1950).

(4) D. M. Brown and B. Lythgoe, *J. Chem. Soc.*, 1990 (1950).

(5) W. Andersen, C. A. Dekker and A. R. Todd, *ibid.*, 2721 (1952).

(6) H. K. Mitchell and F. A. Haskins, *Science*, **110**, 278 (1949).

(7) Dougherty Chem. Co., Richmond Hill, N. Y.

(8) W. Klein, *Z. physiol. Chem.*, **207**, 125 (1932); **255**, 82 (1938).

(9) The recently employed ethanol extraction procedure[5] may be less tedious.

(10) Seed crystals were obtained through the courtesy of Dr. M. S. Dunn of the University of California.

(11) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

(12) N_{260} was defined by Hotchkiss[11] as the absorption of a neutral solution of thymidine (1 mg. per ml.) at 260 mμ. His reported value is 36.

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The Resolution of Parsidol

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Although differences in the pharmacological and physiological effects of the optical isomers of assorted natural products have been well-known for many years,[1] only relatively recently has much attention been given to resolution of synthetic drugs into their enantiomorphs. When the latter has been done it has frequently been found that one of the isomers is more active than the other,[2] although this is not always true.[3]

In order to make available for pharmacological and clinical evaluation both of the optical isomers we have effected a resolution of N-(2-diethylaminopropyl)-phenothiazine,[4] variously known as Parsidol, Lysivane, Ethopropazine, RP3356 and W-483, which has recently shown favorable results[5] in the treatment of Parkinsonism.

Resolution of the racemic base was accomplished with *d*-tartaric acid in *n*-propanol from which solvent the *d*-base *d*-bitartrate crystallized more readily than did the *l*-base *d*-bitartrate.

The pure diastereoisomeric bitartrates were converted to the enantiomorphic *d*- and *l*-bases and thence to the corresponding enantiomorphic hydrochlorides by usual methods. The optical rotation of the hydrochlorides was very small but it was verified that these were indeed the desired *d*- and *l*-salts by their conversion back to the free bases which had the same optical rotations as those obtained from the original *d*-bitartrate.

Pharmacology.—Comparative toxicity determinations of all the salts were made and statistically evaluated. Toxic symptoms following intravenous injection in mice were essentially the same for the *dl*-, *d*- and *l*-base hydrochlorides and for the *d*- and *l*-bitartrates: collapse, exophthalmus, apnea, convulsions and death. Surviving animals were depressed and the respiration was slow. In spite of the general depression, these animals were hyperreactive to minimal stimuli.

The intravenous dose which killed 50% of the mice (LD_{50}) was the same for the racemic and the optically active base hydrochlorides (36 mg./kg.), while the *d*- and *l*-base *d*-bitartrates were less toxic (62 and 54.5 mg./kg.). In terms of the free base, however, only the *d*-base *d*-bitartrate was significantly less toxic (see Table II).

Antagonism of nicotine-induced tremors in the rabbit[6] was used to estimate nicotinolytic activity.

(1) References 3 and 4 on p. 662 and pp. 662–666 of G. L. Jenkins and W. H. Hartung, "The Chemistry of Organic Medicinal Products," Third Edition, John Wiley and Sons, Inc., New York, N. Y., 1949. Some further recent examples are: thyroxine, W. E. Griesbach, T. H. Kennedy and H. D. Purves, *Nature*, **160**, 192 (1947); arterenol, F. P. Luduena, E. Ananenko, O. H. Siegmund and L. C. Miller, *J. Pharmacol. Exptl. Therap.*, **95**, 155 (1949); chloramphenicol, J. Controulis, M. C. Rebstock and H. M. Crooks, Jr., THIS JOURNAL, **71**, 2463 (1949).

(2) Amidone, C. C. Scott, E. B. Robbins and K. K. Chen, *J. Pharmacol. Exptl. Therap.*, **93**, 282 (1948); Dromoran, L. O. Randall and G. Lehmann, *ibid.*, **99**, 163 (1950); Amphetamine, D. F. Marsh and D. A. Herring, *ibid.*, **97**, 68 (1949); other pressor amines, G. M. Badger, *Nature*, **159**, 194 (1947); Lacophenetidide, K. Brand and R. Priesner, *Angew. Chem.*, **62**, 447 (1950); Nirvanol, H. Sobotka, U. S. Patent 1,861,458 (June 7, 1932) (*C. A.*, **26**, 4133 (1932)).

(3) Atabrine, F. A. Bacher, R. P. Buhs, J. C. Hetrick, W. Reiss and N. R. Trenner, THIS JOURNAL, **69**, 1534 (1947).

(4) P. Charpentier (to Société des Usines Chimiques, Rhône-Poulenc), United States Patent 2,526,118 (October 17, 1950); (*C. A.*, **45**, 2511 (1951)).

(5) W. H. Timberlake and R. S. Schwab, *New England J. Med.*, **247**, 98 (1952).

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Although these studies are limited, one can conclude from Table III that the *l*-base, either as the hydrochloride or as the bitartrate, has about twice the nicotinolytic activity of the *d*-base in the form of its corresponding salt.

TABLE I

SUMMARY OF PHYSICAL PROPERTIES OF RACEMIC AND OPTICALLY ACTIVE N-(2-DIETHYLAMINOPROPYL)-PHENOTHIAZINE AND ITS SALTS

Salt	M.p., °C.	$[\alpha]^{25}D$[a]
dl-Bitartrate	135–138	*ca.* +7 to +8[b]
d-Bitartrate	156–158[c]	−4.6[b]
l-Bitartrate dihydrate	93–95[d]	+19[e]
dl-Base	Oil[f,g]	....
d-Base	Oil	+16.7[h]
l-Base	Oil	−16.7[h]
dl-Hydrochloride	220–225[g]	....
d-Hydrochloride	203–210[i,j]	+1[k]
l-Hydrochloride	203–210[i,l]	−1[k]

[a] All rotations observed in a one decimeter tube. [b] 8% in distilled water. [c] *Anal.* Calcd. for $C_{19}H_{24}N_2S \cdot C_4H_6O_6$: C, 59.72; H, 6.54. Found: C, 59.53; H, 6.38. [d] *Anal.* Calcd. for $C_{19}H_{24}N_2S \cdot C_4H_6O_6 \cdot 2H_2O$: C, 55.42; H, 6.82. Found: C, 55.49; H, 6.65. [e] 1% in distilled water. [f] Solidified on standing at room temperature. [g] Prepared and analyzed by P. Charpentier and R. Ducrot, *Compt. rend.*, **232**, 415 (1951). [h] 4% in ethanol. [i] Could be raised to 206–210° by repeated recrystallizations from isopropyl alcohol. [j] *Anal.* Calcd. for $C_{19}H_{24}N_2S \cdot HCl$: N, 8.04; Cl, 10.16. Found: N, 8.02; Cl, 9.94. [k] 6% in ethanol. [l] *Anal.* Calcd. for $C_{19}H_{24}N_2S \cdot HCl$: N, 8.04; Cl, 10.16. Found: N, 7.78; Cl, 9.92.

TABLE II

SUMMARY OF INTRAVENOUS LD_{50} DETERMINATIONS IN MICE OF RACEMIC AND OPTICALLY ACTIVE N-(2-DIETHYLAMINOPROPYL)-PHENOTHIAZINE SALTS

	LD_{50} (mg./kg.)[a]		
Salt	Salt	Calcd. as free base	Limits of error, %
dl-Base hydrochloride	36	32	95–105
d-Base hydrochloride	36	32	90–111
l-Base hydrochloride	35.5	32	88–114
d-Base *d*-bitartrate	62	42	93–108
l-Base *d*-bitartrate	54.5	34	93–108

[a] All solutions were made in physiological saline and were injected at the same rate (0.4 cc./20 g. mouse/10 sec.) by the same worker.

TABLE III

PROTECTION AGAINST NICOTINE-INDUCED TREMORS IN THE RABBIT BY INTRAVENOUS RACEMIC AND OPTICALLY ACTIVE N-(2-DIETHYLAMINOPROPYL)-PHENOTHIAZINE SALTS

	Dose, mg./kg.		No. of animals, protected/no. injected
Salt	Salt	Calcd. as free base	
None	0	0	0/37
dl-Base hydrochloride	2.5	2.24	4/10
	5.0	4.47	9/10
d-Base hydrochloride	2.5	2.24	3/5
	5.0	4.47	5/5
l-Base hydrochloride	1.25	1.12	2/10
	2.5	2.24	5/5
	5.0	4.47	4/5
d-Base *d*-bitartrate	2.5	1.68	1/5
	5.0	3.37	5/5
l-Base *d*-bitartrate	1.79	1.12	4/10
	2.5	1.56	5/5
	5.0	3.13	5/5

We wish to acknowledge the technical assistance of Mr. Heino A. Luts. Microanalyses were carried out by or under the supervision of Dr. F. A. Buehler.

Experimental[7]

***dl*-N-(2-Diethylaminopropyl)-phenothiazine.**—The base was prepared from the corresponding purified hydrochloride, obtained essentially by the procedure of Charpentier,[4] by dissolving the latter in water, basifying the aqueous solution and extracting the resultant oil with ether. Removal of the ether gave the base in quantitative yield.

Resolution of *dl*-N-(2-Diethylaminopropyl)-phenothiazine.—The racemic base (25.0 g., 0.08 mole) and *d*-tartaric acid (12.0 g., 0.08 mole) were dissolved in 300 ml. of warm (50°) *n*-propanol and the solution was allowed to cool slowly. Crystallization was induced by scratching. After remaining 1.5 hours at room temperature the crystals were filtered off and dried *in vacuo*. There was obtained about 13 g. (70%) of partially resolved *d*-base *d*-bitartrate, $[\alpha]^{25}D$ +1°, m.p. 149–151°. The impure *d*-base *d*-bitartrate was purified by three recrystallizations from *n*-propanol. There was obtained 5.9 g. (45%) of pure material, $[\alpha]^{25}D$ −4.6°.

The filtrate from which the impure *d*-base *d*-bitartrate had been removed was allowed to stand for 24–36 hours more at room temperature (25–30°), and deposited crystals that were rich in *l*-base *d*-bitartrate. The crystals were filtered off and dried; they weighed 12 to 15 g. (65–80%) and had $[\alpha]^{25}D$ +14 to +16°. This material could not be purified by recrystallization from *n*-propanol but it was found that the sparingly soluble *l*-base *d*-bitartrate dihydrate could be readily obtained by several recrystallizations of the crude material from water. The pure *l*-base *d*-bitartrate dihydrate obtained from the crude material by recrystallization from three parts of water weighed about 5.4 g. (40%), $[\alpha]^{25}D$ +19°, m.p. 93–95°.

***d*- and *l*-N-(2-Diethylaminopropyl)-phenothiazine.**—Each pure *d*-bitartrate was dissolved in water and the solution was made alkaline with sodium hydroxide and extracted with ether. The ether extract was dried over anhydrous potassium carbonate and the ether removed *in vacuo* leaving the optically active bases as oils. The *d*-bitartrate having $[\alpha]^{25}D$ −4.6° gave a base having $[\alpha]^{25}D$ +16.7° and the *d*-bitartrate having $[\alpha]^{25}D$ +19° gave a base having $[\alpha]^{25}D$ −16.7°.

***d*- and *l*-N-(2-Diethylaminopropyl)-phenothiazine Hydrochloride.**—The optically active bases were dissolved in ether (5% solution) and dry hydrogen chloride passed in until no more precipitate formed. The precipitates were filtered, washed with ether and dried *in vacuo*. The *d*- and *l*-hydrochlorides, $[\alpha]^{25}D$ +1 and −1°, respectively, m.p. 203–210°, were obtained in quantitative yield. Samples of these hydrochlorides were converted back to the free bases in the usual manner; these bases had $[\alpha]^{25}D$ +16.7 and −16.7°, respectively.

(7) Melting points are uncorrected.

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The Preparation of Some Trialkyltin-lithium Compounds

BY HENRY GILMAN AND SANDERS D. ROSENBERG

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In a previous publication[1] from this Laboratory the preparation of triphenyltin-lithium from stannous chloride and three equivalents of phenyllithium was described. In order to ascertain if aromatic groups bonded to the tin atom were necessary for the formation of complexes of this type and investigate the possibility of preparing alkylorganotin compounds by means of similar

(1) H. Gilman and S. D. Rosenberg, THIS JOURNAL, **74**, 531 (1952). See also G. Wittig, F. J. Meyer and G. Lange, *Ann.*, **571**, 167 (1951); G. Wittig, *Angew. Chem.*, **62**, 231 (1950).