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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.

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(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.

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(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.

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