trated by distillation to 300 ml., and rechromatographed on a 3.7 × 25 cm. column of Magnesol packed from dry ethyl acetate. All three compounds, hesperidin, hesperetin and the hesperetin-7-glucoside were present on this column, but a relatively clean separation was possible. The central portion of the middle zone was collected as a separate fraction and taken to dryness under reduced pressure. The resulting solid—yield about 0.35 g.—was purified by four recrystallizations from alcohol. This sample had a m.p. of 206–207° uncor., and an optical rotation $[\alpha]^{27}D$ −51.8° in pyridine, which agree well with those reported for hesperetin-7-glucoside by Zemplén, *et al.*[5] On hydrolysis, the sugar was identified as glucose, with no trace of rhamnose detectable.

**Hydrolysis of Naringin.**—Five grams of naringin, 100 ml. of cyclohexanol and 50 ml. of formic acid were refluxed 20 hr. and then the solvent distilled off until the temperature reached 135°. The first detectable trace of naringenin-7-glucoside was found after 3 hr., and continued to increase relatively for about 15–20 hr. $R_f$ values in 15% acetic acid were 0.80 for naringin; 0.75 for naringenin-7-glucoside; and 0.47 for naringenin. After hydrolysis, the volume of the remaining solution was doubled with acetone and filtered through a 1.5 × 3 cm. column of Magnesol. Filtration removed dark, flocculent material. The solution was then chromatographed on a 7 × 25 cm. column of Magnesol packed as an acetone slurry. When all of the reaction mixture had been adsorbed, a column volume of dry ethyl acetate was passed through to displace the cyclohexanol and then wet ethyl acetate was used to elute the liquid chromatogram. The column was too overloaded to permit detection of zones, but some fractionation was possible. The eluate was collected in fractions and microliter portions were spotted on paper. The first fractions contained mainly naringenin and were discarded. Elution was continued, and fractions containing naringenin-7-glucoside now appeared, and were collected, combined, concentrated to 200 ml. *in vacuo*, and rechromatographed on a 4.5 × 20 cm. column of Magnesol, packed this time with an ethyl acetate slurry. Some separation of the zones was detected under ultraviolet light, but a clear-cut separation was not achieved. The naringenin-7-glucoside fraction from this column was concentrated and again rechromatographed on a fresh 2.5 × 15 cm. column of Magnesol. A good separation between the leading naringenin and the central naringenin-7-glucoside was obtained in this case. A dark, narrow zone appeared at both the top and bottom of the central naringenin-7-glucoside zone. The dark zones were collected separately, and the center of the middle zone was taken as the naringenin-7-glucoside. After evaporation at reduced pressure, the resulting solid—yield about 0.40 g.—was recrystallized several times from methanol–water. The m.p. of the naringenin-7-glucoside was 225–226°, uncor., and no lowering occurred on admixture with a sample of authentic prunin. Mixed paper chromatograms of the prepared naringenin-7-glucoside with prunin showed no separation. $R_f$ values for both were 0.84 in 60% acetic acid–water and 0.92 in the butyl alcohol–acetic acid–water system (4–1–5). The naringenin-7-glucoside and the prunin from the cherry tree[6] are thus identical. After hydrolysis, paper chromatography showed the presence of glucose, but no rhamnose, in the neutralized filtrate.

In preparing a sample of naringenin from naringin, using sulfuric acid, according to the method of Will,[7] paper chromatograms at the end of 2 hr. revealed the presence of some naringenin-7-glucoside. Thus, for naringin hydrolysis, there may be a possibility for using sulfuric instead of formic acid.

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## The Synthesis and Alkaline Decomposition of γ-Aminopropylsulfuric Acid

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The preparation of γ-aminopropylsulfuric acid was undertaken in an effort to convert it into trimethylenimine. The sulfuric ester was first synthesized by Gabriel and Lauer[1] in unreported yields by treating γ-bromopropylamine with silver sulfate. Yields of 90% crude γ-aminopropylsulfuric acid were obtained in this Laboratory by the treatment of γ-aminopropanol with sulfuric acid.

The γ-aminopropylsulfuric acid was subjected to a flash distillation with excess base to give a small yield of impure trimethylenimine.

### Experimental

**γ-Aminopropylsulfuric Acid.**—In a one-liter flask equipped with a stirring motor, thermometer and dropping funnel were placed 50 g. of water and 105 ml. of concentrated sulfuric acid. To this was added dropwise and with cooling a solution containing 150 g. (2 moles) of redistilled γ-aminopropanol and 75 g. of water. The dropping funnel was replaced by a condenser set for downward distillation and the mixture was distilled under slightly reduced pressure until the temperature of the reaction reached 190°. Approximately 140 ml. of water was collected. The mixture was allowed to cool and crystallization was induced by scratching the walls of the flask with a glass rod. The solid cake was then softened with 300 ml. of 95% ethanol, removed from the flask, ground with an additional 400 ml. of ethanol, filtered and dried. The crude γ-aminopropylsulfuric acid (278 g.) was then recrystallized from water. The once recrystallized product melted at 219–220°. Gabriel[1] reported a melting point of 221°. Further recrystallization gave a product which melted at 227–228°.

*Anal.*[2] Calcd. for $C_3H_9C_4NS$: N, 9.02; S, 20.66. Found: N, 8.84; S, 20.54.

**Trimethylenimine.**—The γ-aminopropylsulfuric acid (155 g.) was decomposed in an analogous fashion employed by Reeves[3] for the decomposition of β-aminoethylsulfuric acid. A fraction (1 g.) distilling at 66–69° was obtained. This corresponds to a 1.7% yield of trimethylenimine. The picrate of this fraction melted at 166° and the chloroplatinate at 201°. Gabriel[4] reported melting points for the picrate and chloroplatinate of trimethylenimine at 165° and 200–203°, respectively. The refractive index, however, of the 66–69° fraction was $n^{25}$ 1.3450 which did not correspond to the value observed by Ruzicka[5] for trimethylenimine, *viz.*, $n^{25}D$ 1.4287. The product was considered moist and redried over anhydrous sodium sulfate and redistilled. The refractive index did not change. Further attempts of drying over solid potassium hydroxide and redistillation still gave the same value for the refractive index. A low-boiling fraction 47–64° and a high-boiling fraction 110–191° were also obtained but were not characterized.

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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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