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Configuration of (+)-Dihydrothymine and Related Compounds*

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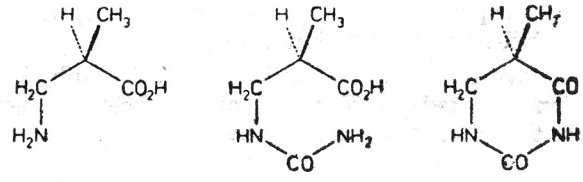
A description of the preparation of (—)-β-ureido*iso*butyric acid (III) and (+)-dihydrothymine (IV) from (—)-β-amino*iso*butyric acid is given. (—)-2,6-Dioxo-4-propylhexahydropyrimidine (VI) was prepared from L-β-amino-*n*-caproic acid.

Optically active III, IV, and VI are new compounds, and evidence is presented for their absolute configuration.

Present knowledge of the metabolic breakdown of pyrimidines mainly originates from studies involving the soil bacillus *Zymobacterium oroticum*[1], and from the investigations of K. Fink *et al.*[2] concerning the fate of uracil and thymine when carried into the organism. Thus dihydrothymine of unknown optical activity has been found as a precursor of β-ureido*iso*butyric acid and of β-amino*iso*butyric acid in products of the metabolic breakdown of thymine.[3] Further, (—)-β-amino*iso*butyric acid (I) has been isolated from human urine,[4] and from bulbs of *Iris tingitana* var. Wedgewood.[5] The synthetic acid (I) has been resolved into optical antipodes, and the (—)-antipode was found to be of the (*R*)-configuration (II).[6]



We have now prepared (—)-β-ureido*iso*butyric acid (III), $[\alpha]_D$-20° (c, 1 in methanol) from (*R*)-β-amino*iso*butyric acid (II) with potassium cyanate. DL-β-Ureido*iso*butyric acid melts at 120—121°, but attempts at crystallizing the (—)-antipode have been unsuccessful, as has also been the case with D- and L-ureidosuccinic acid.[1] Treatment of the acid III with 18% hydrochloric acid afforded (+)-dihydrothymine (IV), $[\alpha]_D$ + 17°. Mild hydrolysis of the thus obtained (+)-dihydrothymine gave the same (—)-β-ureido*iso*butyric acid (III), as was expected. Optically inactive dihydrothymine has been prepared

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earlier by condensation of urea with methacrylic acid,[7] and recently by catalytic hydrogenation of thymine.[8] Infra red spectra of compounds III and IV are presented in Fig. 1.

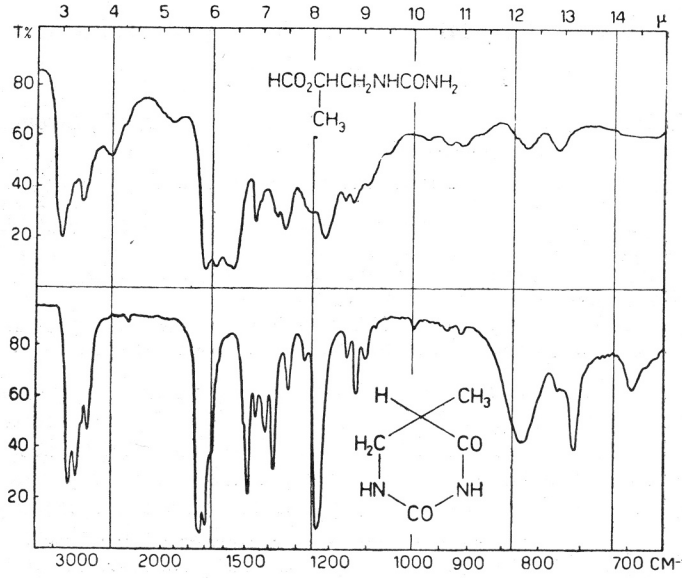
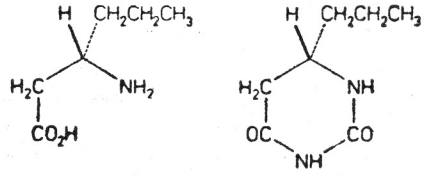


Fig. 1. Infrared absorption spectra of compounds III and IV in potassium bromide plates. Perkin-Elmer Infracord Model 137.

We have also prepared (—)-2,6-dioxo-4-propyl hexahydropyrimidine (VI) from L-β-amino-*n*-caproic acid (V)[9] and potassium cyanate, as described for DL-2,6-dioxo-4-ethyl-hexahydropyrimidine.[10]

From the above reactions it is evident that (+)-dihydrothymine is of the (*R*)-configuration depicted in IV, and that (—)-2,6-dioxo-4-propyl hexahydropyrimidine has the (*S*)-configuration shown in VI.



(+) V (−) VI

Further work on other optically active 2,6-dioxo hexahydropyrimidines is in progress.

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EXPERIMENTAL

All melting points are uncorrected.

(—)-β-*Ureido*isobutyric Acid (III)

A solution of (—)-β-amino*iso*butyric acid (0.98 g., 9.5 mmoles, $[\alpha]_D$—8.5° in water, prepared according to an earlier procedure[6]) and potassium cyanate (1.62 g., 18 mmoles) in water (15 ml.) was heated at 80° for an hour. The reaction mixture was cooled, stirred with Dowex 50 (H form, 12 g.) and filtered. The filtrate was evaporated *in vacuo* and an oily residue (1.1 g.) showing $[\alpha]_D$—13° in methanol was obtained. This oil was dissolved in the minimum quantity of cold methanol and after standing at room temperature for an hour a mixture (150 mg., $[\alpha]_D$—9°, methanol) of both DL- and (—)-β-ureido*iso*butyric acid separated from the solution. The supernatant liquor was evaporated to dryness *in vacuo*, the residue redissolved in a small quantity of methanol and precipitated with absolute ether. The oily precipitate (0.40 g., $[\alpha]_D$—18°, methanol) was dissolved in a mixture of benzene-methanol (1:2, 50 ml.) and chromatographed on alumina (5 g., activity IV according to Brockmann). The column was eluted with the same solvent mixture in fractions of 15 ml. Fractions 5—7 were evaporated to dryness and the residue consisting of (—)-β-ureido*iso*butyric acid (0.26 g.) was precipitated from methanol-ether. The analytical sample was dried at 70°/0.001 mm for 10 hours, and showed $[\alpha]_D$—20.0° ± 0.1° (c, 1.00 in methanol). Transparent, viscous oil.

Anal. 7.651 mg. subst.: 11.489 mg. CO_2, 5.092 mg. H_2O
4.483 mg. subst.: 0.7564 ml. N_2 (20°, 756 mm.)
$C_5H_{10}O_3N_2$ (146.15) calc'd.: C 41.09; H 6.90; N 19.17%
found : C 40.98; H 7.45; N 19.55%

Paper chromatography was carried out on Whatman No. 1 paper, at 22°, using *n*-butanol-ethanol-water (2:1:1, v/v) as mobile phase, and was developed with *p*-dimethylaminobenzaldehyde reagent[11]. The chromatogram showed a yellow spot with *Rf* 0.73 in accordance with earlier findings[11]. The IR-spectrum is given in Fig. 1.

(+)-*Dihydrothymine* (IV)

Cyclization of β-ureido*iso*butyric acid into dihydrothymine was carried out as described in a similar case[21] by refluxing (—)-β-ureido*iso*butyric acid (420 mg. 2.8 mmole, $[\alpha]_D$—12°, methanol) and 18% hydrochloric acid (20 ml.) during two hours. The reaction mixture was cooled, evaporated to dryness *in vacuo*, and a crystalline residue consisting of (+)-dihydrothymine was obtained. Fractional crystallization from ethanol gave 128 mg. of colourless long needles $[\alpha]_D$+5.7° (in pyridine) and from the mother liquors an additional quantity of 210 mg. $[\alpha]_D$+17° (in pyridine) could be isolated. The total yield of dihydrothymine was 92%. The analytical sample was recrystallized twice from methanol and subsequently sublimed at 130°/0.001 mm.; it showed the m.p. 264—265°, and $[\alpha]_D^{21}$ +17° (c, 0.548 in pyridine). DL-Dihydrothymine prepared earlier[7] showed the same m.p.

Anal. 6.861 mg. subst.: 11.864 mg. CO_2, 4.018 mg. H_2O
$C_5H_8O_2N_2$ (128.08) calc'd.: C 46.88; H 6.26%
found : C 47.19; H 6.55%

Paper chromatography showed the same *Rf* values as described earlier for DL-dihydrothymine[11]. The IR-spectrum is given in Fig. 1.

Alkaline Hydrolysis of (+)-*Dihydrothymine to* (—)-β-*Ureido*isobutyric *Acid*

Hydrolysis of (+)-dihydrothymine was carried out in the same manner as described earlier for the DL-compound[18].

(+)-Dihydrothymine (205 mg., 1.6 mmole, $[\alpha]_D$+7°, pyridine) was suspended in water (6.6 ml.), *N*-sodium hydroxide (2.6 ml.) added, and the reaction mixture left

at room temperature for 4 hours. The solution was filtered through a small quantity of Dowex 50 (H form) washed with water, and the combined filtrates evaporated to dryness. Fractional precipitation from methanol-ether gave 87 mg. of oily (—)-β-ureido*iso*butyric acid showing $[\alpha]_D$—12° (in methanol), and characteristic *Rf* values and IR spectrum.

(—)-2,6-Dioxo-4-propyl hexahydropyrimidine

The preparation was carried out in the same manner as in the case of DL-2,6-dioxo-4-ethyl hexahydropyrimidine[10].

A solution of L-β-amino-*n*-caproic acid (655 mg., 5 mmoles, $[\alpha]_D$+50°, methanol, obtained from L-norvaline[9]) and potassium cyanate (810 mg., 10 mmoles) in water (6.5 ml.) was heated at 80° for an hour. The reaction mixture was cooled, acidified with 20% sulphuric acid to *p*H 3.5, 15% hydrochloric acid added (20 ml.), and evaporated to dryness on a water bath. The syrupy residue was triturated twice with a small quantity of water, dried, and recrystallized twice from dioxane-petroleum ether. Colourless needles were obtained in a total yield of 40%, showing the m. p. 194—196°, and $[\alpha]_D^{20}$ —4.2° (c, 1.4 in dioxane). The analytical sample was sublimed at 130°/0.005 mm.

Anal. 7.708 mg. subst.: 15.212 mg. CO_2, 5.495 mg. H_2O
$C_7H_{12}O_2N_2$ (156.18) calc'd.: C 53.83; H 7.75%
found : C 53.85; H 7.98%

REFERENCES

1. I. Lieberman and Kornberg, *J. Biol. Chem.* **207** (1954) 911.
2. K. Fink *et al., J. Biol. Chem.* **197** (1952) 441; **221** (1956) 452.
3. *Cf. e.g.* R. M. Fink, C. McGaughey, R. E. Cline, and K. Fink, *J. Biol. Chem.* **218** (1956) 1.
4. H. R. Crumpler, C. E. Dent, H. Harris, and R. G. Westall, *Nature*, Lond., **167** (1951) 307.
5. S. Asen, J. E. Thompson, C. J. Morris, and F. Irrevere, *J. Biol. Chem.* **234** (1959) 343.
6. K. Balenović and N. Bregant, *Tetrahedron* **5** (1959) 44.
7. E. Fischer and G. Roeder, *Ber.* **34** (1901) 3751.
8. M. Green and S. S. Cohen, *J. Biol. Chem.* **225** (1957) 397.
9. K. Balenović and N. Štimac, *Croat. Chem. Acta* **29** (1957) 153.
10. H. D. Dakin, *J. Biol. Chem.* **99** (1932) 531.
11. R. M. Fink, R. E. Cline, C. McGaughey, and K. Fink, *Anal. Chem.* **28** (1956) 4.
12. S. Gabriel, *Ber.* **38** (1905) 630.
13. R. D. Batt, J. K. Martin, J. McT. Ploeser, and J. Murray, *J. Am. Chem. Soc.* **76** (1954) 3663.

IZVOD

Konfiguracija (+)-dihidrotimina i srodnih spojeva

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Iz (—)-β-amino*iso*maslačne kiseline priređena je (—)-β-ureido*iso*maslačna kiselina (III) i (+)-dihidrotimin (IV). Iz L-amino-*n*-kapronske kiseline priređen je (—)-2,6-diokso-4-propilheksahidropirimidin (VI).

Optički aktivni spojevi III, IV i VI su novi, i daju se dokazi za njihovu apsolutnu konfiguraciju.

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