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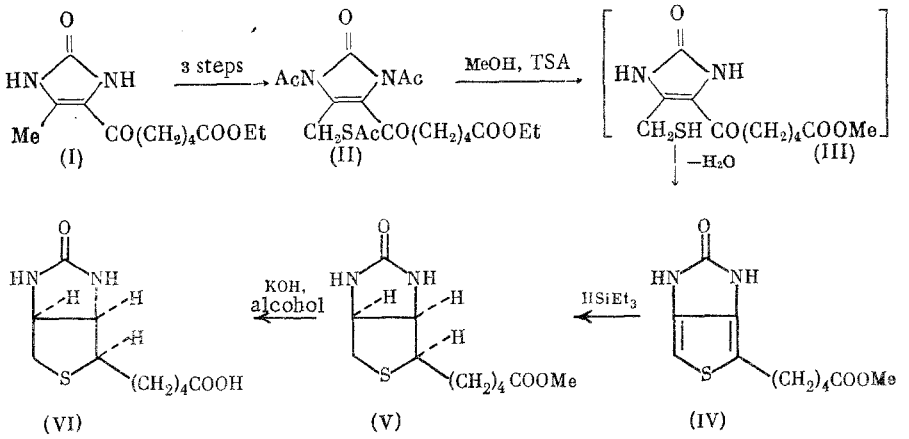
The three-step synthesis of the ethyl ester of N,N'-diacetyl-α-keto-α'-acetylthiodehydrodesthiobiotin (II) from the ethyl ester of α-ketodehydrodesthiobiotin (I) was accomplished previously [1-3]. In this paper we describe the preparative conversion of thioacetate (II) to racemic biotin (VI) by the following scheme:



The methyl ester of 2,3,4,5-tetradehydrobiotin (IV) was obtained in 75% yield by heating thioacetate (II) with MeOH in the presence of p-toluenesulfonic acid (TSA). The N-deacetylation of (II), transesterification, and cyclization of the intermediate mercapto ketoester (III) all occur during reaction [3].

The subsequent cis-hydrogenation of the thiophene ring of ester (IV) using $HSiEt_3$ in CF_3COOH as described in [4], in the presence of BF_3 etherate and alkaline Al_2O_3, at 45-50°C, led to the methyl ester of biotin (V), which after hydrolysis with alcoholic KOH solution gave technical (VI) in 80% yield when based on (IV). Running the hydrogenation of (IV) without the Al_2O_3 lowered the yield of technical (VI) to 40%, and down to 5-8% without the BF_3 etherate.

The growth stimulating activity of technical (VI) toward *Saccharomyces cerevisiae* was 40% of the activity of d-biotin.

Technical (VI) was purified by heating with dilute H_2SO_4 solution, and then with aqueous Na_2CO_3 solution in the presence of resin AV-172 P, followed by recrystallization from 80% alcohol. The yield of the purified (VI) was 50% when based on (IV). The purified (VI) had 50% of the activity of d-biotin when tested on *Saccharomyces cerevisiae* and *Lactobacterium plantarum*. The obtained (VI) coincided with an authentic specimen in its melting point, R_f, and the IR, PMR, and mass spectra.

EXPERIMENTAL

The UV spectrum was taken in alcohol solution on a Specord UV-VIS instrument, and the IR spectrum was taken as a KBr pellet on a UR-20 instrument. The PMR spectra were obtained on a Varian DA-60-IL instrument at an operating frequency of 60 MHz (internal standard = HMDS), and the mass spectrum was obtained on a Varian MAT CH-6 instrument with direct insertion of the substance into the ion source at an ionizing energy of 70 eV and an ionization chamber temperature of 180°. The TLC was run on Silufol UV-254.

Methyl Ester of 2,3,4,5-Tetradehydrobiotin (IV). A solution of 2 g of the Et ester of N,N'-diacetyl-α-keto-α'-acetylthiodehydrodesthiobiotin (II) and 0.2 g of TSA in 10 ml of

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MeOH was refluxed for 4 h, cooled to ∿20°, treated with aqueous Na_2CO_3 solution to pH 7.5-8, and extracted with ethyl acetate (EA). The extract was dried over $MgSO_4$, filtered through either an SiO_2 (∿100 mesh) or an Al_2O_3 (II activity) bed, and evaporated in vacuo. We obtained 0.92 g (75%) of (IV), mp 147-148° (from benzene), R_f 0.32 (EA, the spots were detected either with iodine vapors or in UV light). Ultraviolet spectrum: λ_{max} 260 nm. PMR spectrum (C_5D_5N, δ, ppm): 1.56 m (CH_2CH_2), 2.16 m ($\underline{CH_2}COOCH_3$), 2.63 m ($CH_2C{=}C$), 3.47 s (CH_3O), 6.18 s (HC-C), 11.40 s (2NH).

Racemic Biotin (VI). With stirring, to 20 ml of CF_3COOH were successively added 1 ml of BF_3 etherate, 2 g of (IV), 5 ml of $HSiEt_3$, and 0.2 g of Al_2O_3 (II activity). The mixture was heated for 30 h at 45-50° and then evaporated in vacuo. The residue was treated with 7 ml of conc. HCl in 33 ml of alcohol, let stand for 12 h at ∿20°, and then evaporated in vacuo. With stirring, the residue was treated with a solution of 3.3 g of KOH in 40 ml of alcohol and the mixture was let stand for 12 h at ∿20°. The alcohol was vacuum-distilled, and the residue was dissolved in 10 ml of water and, with cooling (∿0°), was acidified with dilute HCl solution (1:1) to pH ∿1. The obtained precipitate was filtered, washed in succession with water and acetone, and dried in the air. We obtained 1.6 g (80%) of technical (VI), mp 226-227°. A mixture of 1.6 g of technical (VI) and 15 ml of 10% H_2SO_4 solution was refluxed for 1 h. After cooling to ∿20° the precipitate was filtered, washed in succession with water and acetone, and dissolved in a solution of 2 g of Na_2CO_3 in 20 ml of water. To decolorize the obtained solution it was treated in portions with 2 g of resin AV-172 P, heated at the boil for 5 min, filtered hot, and the filtrate was cooled to ∿20° and acidified with dilute HCl solution (1:1). The precipitate was filtered, washed in succession with water and acetone, and dried to constant weight at 100°. We obtained 0.96 g (50%) of pure (VI), mp 231-232° (from 80% alcohol). PMR spectrum (CF_3COOH, δ, ppm): 1.30 m ($CH_2CH_2CH_2$), 2.10 m (CH_2CO), 2.48 m (CH_2S), 2.95 m (CHS), 4.36 and 4.22 m (HC—CH). R_f 0.56 (EA—alcohol, 3:2, the spots were detected first with $K_3Fe(CN)_6$ solution and then with iodine vapors). Mass spectrum, m/z: 244 (M^+), 184, 112, 97, 85.

The authentic (VI) racemate had the same characteristics.

The paper is dedicated to Prof. E. Lederer (France) on the occasion of his 75th birthday.

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CONCLUSIONS

Starting with the ethyl ester of N,N'-diacetyl-α-keto-α'-acetylmercaptodehydrodesthiobiotin, the three-step synthesis of racemic biotin was accomplished in 37% yield.

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