Further Studies on the Chemical Structure of "Hiochic Acid"

Sir:

In the previous paper\(^1,2\) the author reported the presumed structures of hiochic acid, a new growth factor for *Lactobacillus homohiochii* and *Lactobacillus heterohiochii*. The acid was isolated in crystalline forms, from the culture broth of *Aspergillus oryzae*, as quinine salt (mp. 137-8\(^\circ\)C) or S-benzylthiuronium salt (122-3\(^\circ\)C).

The lactone of hiochic acid is a (3-hydroxy-6-lactone having a formula of C\(_6\)H\(_{10}\)O\(_3\) and the following four structures (I-IV) are compatible with the known facts.

1. \[\text{CHOH} \quad \text{CH}_3 \quad \text{CH} \quad \text{CH} \quad \text{O}\]
2. \[\text{CH}_3 \quad \text{CHOH} \quad \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \quad \text{O}\]
3. \[\text{CH}_3 \quad \text{CO} \quad \text{CH} \quad \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \quad \text{O}\]
4. \[\text{CH}_3 \quad \text{CO} \quad \text{CH} \quad \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \quad \text{O}\]

In the previous report\(^3\), it was supposed that \(\text{HOH-8-caprolactone (I)}\) seemed to be most probable, in view of the fact that the iodoform formation test was positive for natural hiochic acid. However, some corrections of the previous view were necessary from the result of synthesis of hiochic acid and its related compounds.

The \(\alpha,\beta\)-unsaturated acid resulted from hiochic acid by the treatment with 10% H\(_2\)SO\(_4\), showed a definite peak of absorption at 222 \(\mu\) (\(\varepsilon=10,000\)), while the synthetic parasorbic acid\(^3\) (V) (b.p. 95-100°/12 mm, \(n^\text{B}=1.476\)) prepared from \(\beta,\beta\)-dibromocaproic acid, showed only an end absorption (\(\varepsilon=10,000\) at 208 \(\mu\)).

This fact obviously shows that the former is different from parasorbic acid, therefore the presumption of structure I for hiochic acid must be excluded.

As the structure III is also compatible with most of the known characteristics of natural hiochic acid, \(\beta\)-hydroxy-\(\beta\)-methyl-\(\delta\)-valerolactone (III) was synthesized from \(\beta\)-hydroxy-\(\beta\)-methylglutaric aldehyde by the Tischenko reaction using aluminium isopropoxide. The \(\beta\)-hydroxy-\(\beta\)-methylglutaric aldehyde was prepared from diallylmethylcarbinol. The synthetic lactone thus obtained, was purified by silicagel chromatography and repeated distillation in vacuo.

**Anal.** Calculated for C\(_6\)H\(_{10}\)O\(_3\): C, 55.37%; H, 7.75.

**Found:** C, 55.51%; H, 7.75.

Lactone of natural b.p. 130-35°/2 mm \(n^\text{B}=1.474\)

**Hiochic acid** b.p. 145-50°/5 mm \([\alpha]_D^{20}=19.9°\) (in \(95\%\) ethanol)

Synthetic \(\beta\)-hydroxy-\(\beta\)-methyl-\(\delta\)-valerolactone

The infra red spectra of the synthesized \(\beta\)-hydroxy-\(\beta\)-methyl-\(\delta\)-valerolactone both in liquid film and in chloroform solution were entirely identical with natural hiochic acid (Figs. 1 and 2). The iodoform formation from the synthesized lactone with alkaline iodine also proved to be distinctly positive.

The biological activity of the lactone for *Lactobacillus heterohiochii* was one half that of natural lactone. From these facts, it is concluded that the lactone of hiochic acid is identical with \(\beta\)-hydroxy-\(\beta\)-methyl-\(\delta\)-valerolactone and that only the levorotatory isomer of the lactone is biologically active.

During the course of this study, Skeggs

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et al isolated a new acetate replacing factor for *Lactobacillus acidophilus* and reported that the factor was identified as \( \beta \)-hydroxy-\( \beta \)-methyl-\( \delta \)-valerolactone (III). They synthesized this lactone by partial reduction of \( \beta \)-hydroxy-\( \beta \)-methylglutaric acid and named it divalonic acid. The iodoform formation test of their factor was reported to be negative. However, it was confirmed by the present author that the synthetic divalonic acid, which was kindly supplied from Dr. K. Folkers, was also biologically active for the growth of our test organism, *Lact. heterohiochii* which does absolutely require hiochic acid. The activity of synthetic divalonic acid was also one half that of natural hiochic acid as, likely as was the lactone synthesized by the present author. It is apparent, therefore, that the chemical structure of hiochic acid is identical with that of divalonic acid.

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