Crude bios(1) was treated with a strong (30%) caustic soda solution, at boiling temperature, for 3 hours and to the reaction mixture a large amount of pure alcohol was added. On standing over night, a resinous matter separated out this being easily removed by decantation. The clear solution thus obtained was submitted to distillation under reduced pressure until its volume about one half of the original. On standing over one night or more, yellowish crystalline plates separated out, proving to be sodium salt of an organic acid. The free acid, formed by adding dilute sulfuric acid to the aqueous solution

STUDIES ON BIOS. PART III.

By

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of the salt, was recrystallized from either hot water or benzene. This free acid contains neither S nor N and has the experimental formula of $C_{10}H_{10}O_5$ (m. w. found 216), to which we have given the name of $\gamma$-acid, for the time being, and it contains one carboxyl and hydroxyl and two methoxy groups. The acid itself is quite unstable but its methylated products become durable for oxidation by alkaline permanganate solution at room temperature, forming trimethyl gallic acid (I). In this respect it resembles the iridic acid ($C_{10}H_{12}O_5$) (II) of G. de Laire and Tiemann. It might be suspected, therefore, that the organic acid is an isomeride of iridic acid.

In order to orientate the hydroxyl of $\gamma$-acid, it was converted to ethyl derivative, using ethyl iodide, which was subsequently oxidized with alkaline permanganate, to ethyl dimethyl gallic acid (IV). This has been proved, on the other hand, to be identical with 3, 5-dimethyl-4-ethyl gallic acid, so that it has been established that hydroxyl of $\gamma$-acid is on 4 position while that of iridic acid is on 5 to the main side chain.

If $\gamma$-acid is an isomeride of iridic acid, differing only in the relative positions of the hydroxyl and methoxy groups, the methyl derivatives of both substances must be identical. But this is not the case and, moreover, the following facts. i. e. that the results of elementary analysis of $\gamma$-acid does not conform to the formula of $C_{10}H_{12}O_5$; that methyl $\gamma$-acid does not form a stable dibromo compound while iridic acid does; that on dry distillation methyl iridic acid converts to 3, 4, 5-trimethoxy-1-methyl benzene, which is not the case with $\gamma$-acid and that the methyl ester of methyl iridic acid is an oil while that of $\gamma$-acid is a crystalline substance, show that $\gamma$-acid differs also from iridic acid in regard to the form of other elementary groups.

By the action of hydroxylamine on $\gamma$-acid in acidic medium an oxime (V) is formed; the reaction, however, does not take place in an alkaline solution. It was assumed, therefore, that there are two tautomeric isomerides, keto-and enol-form, in $\gamma$-acid, in alkaline solution converting to enol-form.

Taking the following facts into consideration, i. e. that $\gamma$-acid is a coloring matter: that one hydroxyl, which lies on $p$-position to the main side chain, easily changes to ketone group and that, to conform with the results of analysis, one of two carbon atoms of the main side chain cannot take more than one atom of hydrogen, we assigned quinoid ring to $\gamma$-acid. (VI).

If $\gamma$-acid has the constitution of VI, it should be expected to obtain glyoxylic acid and dimethyl gallic acid or their derivatives, by acid hydrolysis. In fact formaldehyde was identified as one of the products, while an oily matter, which is considered to be a condensation product of quinoid substance or dimethyl pyrogallol, was found in the residue. Formaldehyde was produced, presumably, by the decomposition of glyoxylic acid.
Experimental

γ-acid. To 20 g. of crude bios 100 c.c. of a 30% caustic soda solution was added and kept at boiling temperature for 3 hours. The reaction mixture was filled up to 1 lt. with pure alcohol. On standing over night a resinous matter separated out which was removed by decantation. The clear solution thus obtained was submitted to distillation under reduced pressure until its volume became about one half of the original when yellowish brown crystalline plates have separated out. The free acid obtained by treating the salt with sulfuric acid was recrystallized either from hot water or benzene. M. p. : 187-189° (decomposing). m. w. found : 235 (by m. p. depression of camphor solution). Subst. : 0.0512 g., CO₂ : 0.1074 g., H₂O : 0.0226 g., C : 57.20%, H : 4.90% (C : 57.13%, H : 4.76% as C₁₀H₁₀O₅).

Benzoyl γ-acid. γ-acid was benzoylated by Schotten-Baumann’s method using benzoyl chloride. M. p. : 216°, subst. : 0.0544 g., CO₂ : 0.1300 g., H₂O : 0.0232 g., C : 65.17%, H : 4.73% [C : 64.96%, H : 4.45% as C₁₀H₈O₅(C₆H₅CO)].

Methyl γ-acid. When γ-acid was methylated with di-methyl sulfate, 2 forms were produced. The one, which is neutral to litmus, separated out from a neutral medium. M. p. : 100°C, subst. : 0.0498 g., CO₂ : 0.1096 g., H₂O : 0.0264 g., C : 60.02%, H : 5.89% [C : 60.50%, H : 5.88% as C₉H₈O₂(OCH₃)(COOCH₃)].

The other was an acid which separated out from an acidic medium. M. p. : 130°C, subst. : 0.0505 g., CO₂ : 0.1104 g., H₂O : 0.0234 g., C : 59.62%,
H: 5.14% [C: 58.92%, H: 5.35% as C\textsubscript{18}H\textsubscript{20}O\textsubscript{4} (OCH\textsubscript{3})].

**Ethyl ester of γ-acid.** The ester was prepared by dissolving γ-acid in pure alcohol and passing dry HCl. M. p.: 86° C, subst.: 0.0488 g., CO\textsubscript{2}: 0.1094 g., H\textsubscript{2}O: 0.0208 g., C: 61.14%, H: 6.10%. [C: 60.50%, H: 5.88% as C\textsubscript{8}H\textsubscript{9}O\textsubscript{4} (COOC\textsubscript{2}H\textsubscript{5})].

**Trimethyl gallic acid.** 1g. of methyl γ-acid was dissolved in a 0.5% caustic soda solution and, under a thorough cooling and constant stirring, KMnO\textsubscript{4} solution (5%) was added drop by drop. Mangan oxide formed was filtered off, the filtrate was concentrated by distillation under reduced pressure and the solution was acidified when the substance crystallized out. M. p.: 169° C, subst.: 0.0469 g., CO\textsubscript{2}: 0.0976 g., H\textsubscript{2}O: 0.0242 g., C: 56.75%, H: 5.73%, [C: 56.58%, H: 5.65% as (CH\textsubscript{3}O)\textsubscript{3} C\textsubscript{6}H\textsubscript{2} COOH].

Trimethyl gallic acid prepared synthetically from gallic acid had the same m. p. and the mixture of the two also melted at the same temperature.

**Dry distillation of γ-acid.** An oily substance was obtained by dry distillation of methyl γ-acid under reduced pressure. It decomposes at about 330° C without boiling, differing from 3, 4, 5, trimethoxy-1-methyl benzene obtainable by the similar treatment of iridic acid. By oxidizing the substance with alkaline KMnO\textsubscript{4}, trimethyl gallic acid was obtained.

**Ethyl γ-acid.** 2g. of γ-acid was dissolved in 30c.c. of 5% caustic soda, an excess of ethyl iodide was added to the solution and the reaction mixture was heated for 5 hours. The solution was cooled and was made acidic by adding sulfuric acid when colourless crystals separated out. Subst.: 0.0513 g., CO\textsubscript{2}: 0.1133 g., H\textsubscript{2}O: 0.0290 g., C: 60.23%, H: 6.27% [C: 60.50%, H: 5.88% as C\textsubscript{18}H\textsubscript{20}O\textsubscript{4} (OC\textsubscript{2}H\textsubscript{5})].

3, 5-dimethyl-4-ethyl gallic acid. Ethyl γ-acid was oxidized as methyl γ-acid. M. p.: 123° C, subst.: 0.0478 g., CO\textsubscript{2}: 0.1014 g., H\textsubscript{2}O: 0.0286 g., C: 57.85%, H: 6.64% [C: 58.40%, H: 6.19% as (CH\textsubscript{3}O)\textsubscript{2} (C\textsubscript{2}H\textsubscript{5}O) C\textsubscript{6}H\textsubscript{2} COOH].

Syringaic acid (3, 5-dimethyl gallic acid) was synthesized by the method of Graebe and Martz and was ethylated with ethyl iodide, the effect being that the product (3, 5-dimethyl ethyl gallic acid) was proved to be the same substance as that obtained from ethyl-γ-acid showing the same composition, and the same m. p. No alteration of the m. p. by mixing the two, was observed.

**Dihydro γ-acid.** γ-acid was reduced in an alcoholic solution with Willstätter's method, and dihydro-γ-acid was obtained. M. p.: 102° C, subst.: 0.0511 g., CO\textsubscript{2}: 0.105 g., H\textsubscript{2}O: 0.0256 g., C: 56.52%, H: 5.56% [C: 56.58%, H: 5.70% as C\textsubscript{18}H\textsubscript{20}O\textsubscript{4}].

**Dihydro-methyl-γ-acid.** Dihydro-γ-acid was oxidized with alkaline per-
manganate. M. p.: 105°C, subst.: 0.0493g., CO₂: 0.1058g., H₂O: 0.0271g., C: 58.52%, H: 6.10% [C: 58.40% as C₁₀H₁₁O₅•CH₃].

This substance is quite stable against any oxidizing agent alkali permanganate showing no trace of oxidation.

Dihydro dibromo methyl γ-acid. Dihydro methyl γ-acid was dissolved in glacial acetic acid and was brominated. M. p.: 122°C, subst.: 0.1005g., AgBr: 0.0984g., Br: 41.67% [Br: 41.63% as C₁₁H₁₂O₅Br₂].

Oxime. 3g. of γ-acid, 2.5g. of hydroxylamine hydrochloride and 3.5g. of sodium acetate were dissolved in 80 c.c. of 60% alcohol and the solution was heated for 3 hours on a water bath. Colorless plates separated out. The substance had no melting point, decomposing at 199–201°C. N: 5.95% [6.22% as C₁₀H₁₁O₅N].

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ON THE REVERSIBILITY OF ENZYME ACTION.

PART I.

By

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Armstrong(1) found that, by the action of yeast maltase on glucose, isomaltose mainly was formed and has taken the view that an enzyme synthesizes exactly those bodies which it does not hydrolyse. To accept this view, it is a matter of primary importance to ascertain whether the maltase preparation used by Armstrong really did not contain any β-glucosidase. On reading Armstrong's papers, one cannot get any information about this matter either positively or negatively.

The observation of the presence of β-glucosidase in yeast cells (brewer's yeast), made by Henry and Auld,(2) is well founded and Pringsheim(3) noticed that maltose and a β-glucoside are formed by the action of yeast enzymes. It is very likely that these experiments present an argument against Armstrong's view.