lerolactone is either the important biological precursor of isoprene units, or is converted by some relatively minor biochemical transformation to an "active isoprene" unit. In either case, this compound offers promise in the study not only of steroid biogenesis but also of other natural substances that arise completely or in part by the condensation of isoprenoid units, *e.g.*, the carotenes, the tocopherols and rubber.

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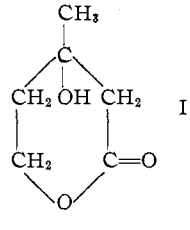
β-HYDROXY-β-METHYL-δ-VALEROLACTONE (DIVALONIC ACID), A NEW BIOLOGICAL FACTOR

Sir:

A new acetate-replacing factor for lactobacilli has been identified by structural degradation and synthesis as β-hydroxy-β-methyl-δ-valerolactone (I). The discovery of this substance and its preparation in highly purified form has been described.[1,2] In aqueous solution, the substance gave an acidic reaction. The potentiometric titration curve rose sharply on addition of alkali, and then drifted in the manner characteristic of lactones. A back-titration of the alkaline solution gave a typical neutralization curve with an equivalent weight of 128 and a *p*H $^1/_2$ value of 4.3 which indicates an acid strength intermediate between that of an unsubstituted carboxylic acid such as acetic acid and a stronger α-hydroxy acid such as lactic acid.

The infrared spectrum of the substance in chloroform gave clear evidence for the presence of hydroxyl-function (2.90–2.95 μ) and δ-lactone function (5.78 μ), but no indication of a carboxyl group. When the substance was dissolved in morpholine and its infrared spectrum recorded at intervals over a period of forty-eight hours, the band ascribed to the δ-lactone function (5.78 μ) slowly decreased in intensity while a band due to carboxyl-function (6.1 μ) appeared and then increased.

Reactions were carried out on the factor with acetic anhydride and benzoyl chloride but non-crystalline products were obtained; reaction with *p*-nitrobenzoyl chloride and pyridine gave decomposition.



I

Amides were formed with ammonia and benzylamine, but they were not obtained crystalline. However, a crystalline amide was obtained with benzhydrylamine which could be used for final critical purification and for analysis; m.p. 92–93°, $[\alpha]^{20}D$ −2.0° (*c* = 20 mg./ml. in ethanol). (Calcd. for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found: C, 72.70, 72.60; H, 7.17, 7.07; N, 4.74.) Analysis for the presence of a C-methyl group in the benzhydrylamide gave results suggestive of one terminal methyl group (calcd. for 1 C-methyl, 4.80. Found: C-methyl, 5.9).

Both the lactone and its benzhydrylamide were examined for the presence of adjacent hydroxyl groups by reaction with alkaline periodate. No periodate was consumed, which indicated that a glycol structure was not present. With alkaline iodine no iodoform was produced from either the lactone or its benzhydrylamide which indicated the absence of the grouping: CH_3CHOH.

Acetylation of the benzhydrylamide with acetic anhydride in pyridine gave a monoacetate; m.p. 104–105°, $[\alpha]^{20}D$ +1.6° (*c* = 45 mg./ml. in ethanol) (calcd. for $C_{21}H_{25}NO_4$: C, 70.97; H, 7.09; acetyl, 12.1. Found: C, 70.70, 70.80; H, 7.09, 6.87; acetyl, 11.4).

The structure β-hydroxy-β-methyl-δ-valerolactone (I) is compatible with these known facts. This structure was confirmed by synthesis. Partial reduction of β-hydroxy-β-methylglutaric acid, a compound previously known as a possible precursor of sterols,[3,4] gave DL-β-hydroxy-β-methyl-δ-valerolactone. The benzhydrylamides of the synthetic DL-lactone and the factor prepared from distillers solubles had identical infrared spectra. Hydrolysis of the synthetic benzhydrylamide gave the DL-lactone which was one-half as active microbiologically as the factor prepared from distillers solubles.

The generic name divalonic acid is being used to designate the corresponding β,δ-dihydroxy-β-methylvaleric acid. The function of divalonic acid in steroid synthesis is presented in the accompanying paper.[5]

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