STUDIES ON AMINO-ACIDS AND RELATED COMPOUNDS. PART IV. ELECTROLYTIC REACTIONS OF LEUCIC ACID.

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l-Leucic acid is one of the physiologically interesting hydroxy-acids and has a close relation with leucine. It is easily obtained by the action of nitrous acid upon natural leucine. H. D. Dakin(1) has described briefly that leucic acid when oxidised with hydrogen peroxide yielded acetone, isovaleraldehyde, and a mixture of acids which were probably isovaleric acid and isobutyric acid. Fr. Fichter and Fr. Kuhn(2) reported that leucine gave several oxidation products, when its solution in sulphuric acid was electrolysed with platinum electrodes.

The author undertook the present work with the intention of comparing the oxidation products of leucic acid with those of leucine. Leucic acid in N.-sulphuric acid was electrolysed in an undivided cell with a lead peroxide anode and a lead cathode. During the progress of the electrolysis, a yellowish oily layer separated out on the surface of the electroly-

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(1) H. D. Dakin, *J. biol. Chem.*, **4** (1908), 91–100.

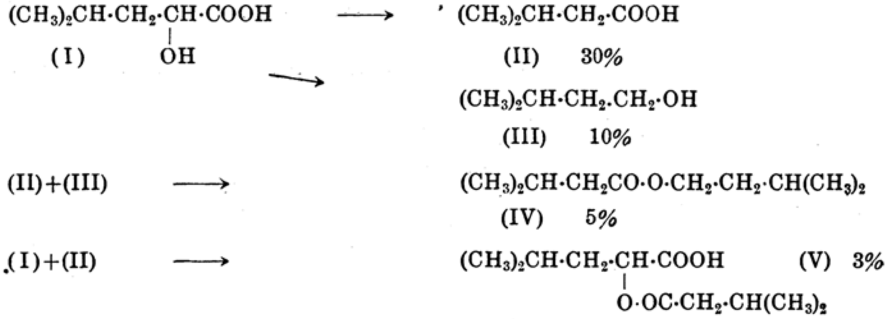
(2) Fr. Fichter and Fr. Kuhn, *Helv. chim. Acta,* **7** (1924), 167.

sate. It was found that the oily layer consisted of isovaleric acid (10%), isovaleryl-leucic acid (3%) and isoamyl ester of isovaleric acid (5%). From the aqueous solution isoamyl alcohol (5%) and isovaleric acid (20%) as well as unchanged leucic acid were isolated. In short, the oxidation products were isovaleric acid (II), isoamyl alcohol (III), isoamyl isovalerate (IV) and isovaleryl-leucic acid (V), but the lower fatty acids were not found. The gaseous oxidation products were not examined.

It is known that leucine is vigorously attacked by anodic oxidation and yields isovaleraldehyde, acetone, isovaleric, isobutyric, acetic and formic acids, as well as ammonia and carbon dioxide. As mentioned above, the oxidation products of leucine and those of leucic acid vary from each other; and it may be concluded that they behave quite differently on oxidation, though they only differ at their α-substitution group, the former having amino and the latter having hydroxyl group.

Identification of the oxidation products: Amyl alcohol was identified by conversion into isovaleric acid and into crystalline isoamyl α-ester of 3-nitrophthalic acid,⁽³⁾ m.p. 164–165°C. To identify isovaleryl-leucic acid, it was hydrolysed with alkali and the two components formed were isolated. The boiling point of this acyl-oxy-acid is concordant with that of the synthetic sample, which was prepared from leucic acid and isovaleryl chloride.

The electrolytic reaction of leucic acid may be briefly represented as follows (excepting the gaseous products):



$$\begin{array}{lcl}
(CH_3)_2CH\cdot CH_2\cdot \underset{\displaystyle OH}{\underset{|}{C}}H\cdot COOH & \longrightarrow & (CH_3)_2CH\cdot CH_2\cdot COOH \\
\quad (I) & & \quad (II) \quad 30\% \\
 & \longrightarrow & (CH_3)_2CH\cdot CH_2.CH_2\cdot OH \\
 & & \quad (III) \quad 10\% \\
(II)+(III) & \longrightarrow & (CH_3)_2CH\cdot CH_2CO\cdot O\cdot CH_2\cdot CH_2\cdot CH(CH_3)_2 \\
 & & \quad (IV) \quad 5\% \\
(I)+(II) & \longrightarrow & (CH_3)_2CH\cdot CH_2\cdot \underset{\displaystyle O\cdot OC\cdot CH_2\cdot CH(CH_3)_2}{\underset{|}{C}}H\cdot COOH \quad (V) \quad 3\%
\end{array}$$

The similar examples of the formation of esters during electrolysis are frequently met with in the literature.⁽⁴⁾ Isoamyl alcohol might be formed by the cathodic reduction of isovaleryl aldehyde.

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(3) This ester is easily obtained from its components when heated together, and it seems to be a suitable compound for identification of isoamyl alcohol.

(4) P. Askenasy, R. Leiser und N. Grünstein, *Z. Electrochem.*, **15** (1909), 846, 852. C. J. Brockman, "Electro-organic Chemistry," (1926) p. 81, 84.

Experimental Part.

Apparatus. Cell: A cylindrical glass vessel (dia. 6 cm., height 9 cm.) provided with a rubber stopper carrying a condenser, a thermometer and two electrodes. Anode (4×5 cm.): lead peroxide sheet; cathode (4×5 cm.): lead sheet, the distance between anode and cathode was 1.5 cm.

l-Leucic acid $(CH_3)_2CH.CH_2.CH(OH).COOH$, m.p. 78°C., was prepared from natural *l*-leucine and nitrous acid. 13.20 Gr. (1/10 mol) of leucic acid were dissolved in 120 c.c. of N.-sulphuric acid and electrolysed in an undivided cell, under the following conditions, C. D.: 2 amp./dm^2., temperature: 38.5°C. quantity of electricity: 12.0 amp. hours.

Separation of Oxidation Products. During the progress of the electrolysis an oily layer separated on the surface of the electrolysate. The oily layer was treated with a saturated solution of sodium bicarbonate, and extracted with ether to separate the neutral part (B) from the acidic part (A). The sodium bicarbonate solution of the latter was acidified with sulphuric acid and submitted to steam distillation, the distillate (A_1) was neutralised with sodium hydroxide, evaporated to dryness and distilled after adding phosphoric acid. The distillate was redistilled and separated into (1) the first running (165–172°C.) and (2) the main fraction (172–174°C.). Each fraction was converted into silver salt and analysed.

Anal.: Subst. (1) = 0.2241; Ag = 0.1154 gr.
„ (2) = 0.3080; Ag = 0.1593 gr.
Found: Ag (1) = 51.52; Ag (2) = 51.71%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

From these analyses and boiling point, it may be said that the fraction (1) as well as the fraction (2) consisted of isovaleric acid without lower members of fatty acids.

After the steam distillation of (A), a portion of the oil (A_2) separating in the flask was converted into silver salt and analysed. Thus the oil (A_2) was speculated as an acyl-oxy-acid formed from isovaleric and leucic acids, and the attempt of distillation under reduced pressure was successful. The oil passed over at 165–175°C. under 24 mm. with slight decomposition. Another portion of this oil was heated with concentrated solution of sodium hydroxide on the water bath for several hours and distilled in steam. From this distillate isovaleric acid was obtained.

Anal.: Subst. = 0.2608; Ag = 0.1346 gr. Found: Ag = 51.60%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

The residue of steam treatments was extracted with ether. After removal of ether, the crystals formed were identified as leucic acid.

Anal.: Subst. = 0.2023; Ag = 0.0912 gr. Found: Ag = 45.08%.
Calc. for $C_6H_{11}O_3Ag$ (silver leucinate); Ag = 45.14%.

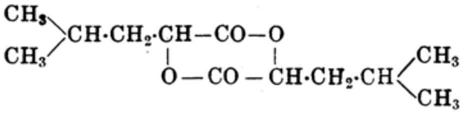
As the distillate was contaminated with its decomposition products, it was purified by steam treatment and by washing with water, then it was converted into silver salt.

Anal.: Subst. = 0.2203; Ag = 0.0728 gr. Found: Ag = 33.05%.
Calc. for $C_{11}H_{19}O_4Ag$ (isovaleryl-leucic acid): Ag = 33.41%.

To establish a positive proof, the same compound was synthesised from isovaleryl chloride (1.5 mol) and leucic acid (1 mol) by heating on the water bath. The reaction product distilled at 165–175°C. / 24 mm. The distillate was treated as above and its silver salt was analysed.

Anal.: Subst. = 0.3342; Ag = 0.1110 gr. Found: Ag = 33.21%.
Calc. for $C_{11}H_{19}O_4Ag$ (isovaleryl-leucic acid): Ag = 33.41%.

During the distillation of this acid a small quantity of crystals separated out from the distillate, which were identified as di-isobutyl glycolide (the dehydration product of leucic acid), m.p. 169–170°C., by comparison with a pure synthetic specimen.



Anal.: Subst. = 0.0935, 0.1125; CO_2 = 0.2164, 0.2614;
H_2O = 0.0699, 0.0844 gr.
Found: C = 63.16, 63.37; H = 8.36, 8.39%.
Calc. for $C_{12}H_{20}O_4$ (di-isobutyl glycolide): C = 63.11; H = 8.83%.

Neutral Part (B). The residue, after removal of ether, gave no aldehyde and ketone reaction. It was distilled under ordinary pressure, two fractions being obtained, b. p. 130–140°C. (1.2 gr.) and 190–195°C. (1.1 gr.). The former fraction smelt of amyl alcohol and was identified as such by conversion into isovaleric acid and by the separation of mono-amyl α-ester of 3-nitrophthalic acid, m. p. 164–165°C.

Oxidation of amyl alcohol to isovaleric acid: 0.7 gr. of alcohol was oxidised with a mixture of 3 gr. $K_2Cr_2O_7$, 5 gr. H_2SO_4 and 15 c.c. H_2O, alcohol being converted into acid (4.86 c.c. of N.-NaOH).

Anal.: Subst. = 0.2712; Ag = 0.1406 gr. Found: Ag = 51.83%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

Nitrophthalic acid ester⁽⁵⁾: The crystalline mass formed by heating the mixture of 1 gr. of alcohol and 2 gr. of 3-nitrophthalic anhydride was recrystallised from benzene in yellow crystals melting at 164–165°C.

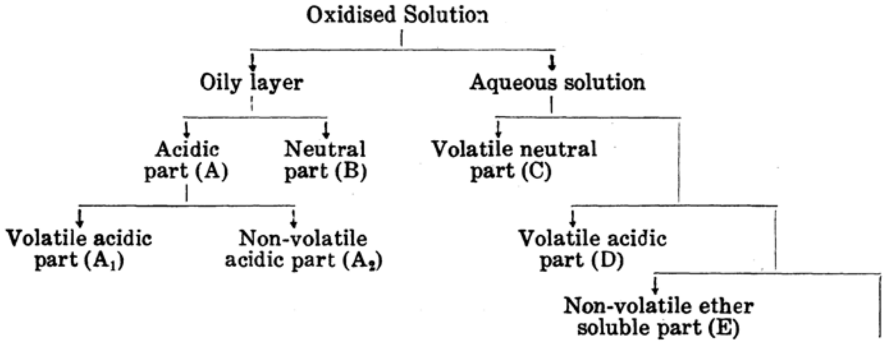
Anal.: Subst. = 0.1039, 0.1092; CO_2 = 2127, 0.2236;
H_2O = 0.0489, 0.0529 gr.
Found: C = 55.84, 55.85; H = 5.27, 5.42%.
Calc. for $C_{13}H_{15}O_6N$ (monoisoamyl ester of 3-nitrophthalic acid): C = 55.49;
H = 5.38%.

The fraction (190–195°C.) on hydrolysis with 40% NaOH yielded isovaleric acid and amyl alcohol. The former was identified by its silver salt.

Anal.: Subst. = 0.4212; Ag = 0.2159 gr. Found: Ag = 51.25%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

The latter was identified as amyl alcohol by conversion into acid as described above.

Anal.: Subst. = 0.1373, Ag = 0.0710 gr. Found: Ag = 51.71%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.



Aqueous Solution. The aqueous part was neutralised with sodium hydroxide and submitted to steam distillation to separate the neutral volatile part (C). From (C) amyl alcohol was obtained, but no ester was found. The former was identified as usual.

Anal.: Subst. = 0.2628; Ag = 0.1356 gr. Found: Ag = 51.57%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

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(5) MacKenzie, *J. Chem. Soc.*, **79** (1901), 1135.

The residue of (C) was again distilled with steam, after acidifying with sulphuric acid to separate volatile acidic part (B). (D) gave only isovaleric acid in considerable amount (20%).

Anal.: Subst. = 0.3171; Ag = 0.1632 gr. Found: Ag = 51.46%.
Calc. for $C_5H_9O_2Ag$ (silver isovalerate): Ag = 51.64%.

After the separation of (D), the solution was again neutralised, concentrated and extracted with ether. From the ethereal extract unchanged leucic acid was recovered (E).

Anal.: Subst. = 0.3673; Ag = 0.1650 gr. Found: Ag = 44.94%.
Calc. for $C_6H_{11}O_3Ag$ (silver leucinate): Ag = 45.14%.

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