SYNTHESIS OF 2,3-DIHYDROXY-3-METHYLVALERIC ACID

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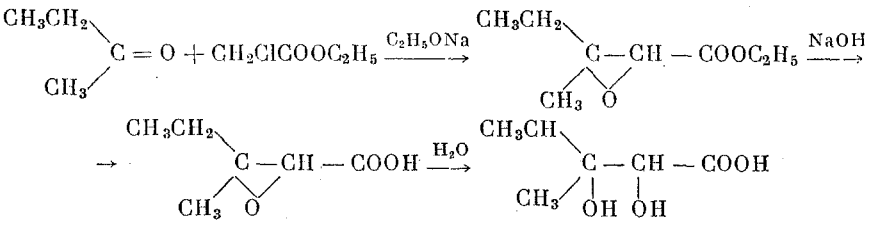
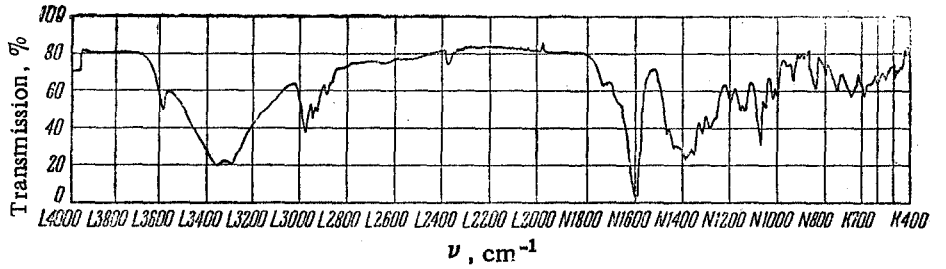
Translated from Izvestiya Akademii Nauk SSSR, Otdelenie Khimicheskikh Nauk,
No. 2, pp. 317-320, February, 1962
Original article submitted September 18, 1961

While studying the mechanism of the biosynthesis of isoleucine in green vegetation in V. L. Kretovich's laboratory, we found it necessary to have various nonnitrogenous analogs of this amino acid at our disposal, in particular the keto analog (3-methyl-2-oxovaleric acid) and the 2,3-dihydroxy analog (2,3-dihydroxy-3-methylvaleric acid). The keto analog of isoleucine was synthesized by a method described by us earlier [1]. For the synthesis of the dihydroxy analog of isoleucine we used the method of Claisen and Darzens as modified by us. The method is based on the condensation of chloroacetic ester with butanone in presence of sodium ethoxide with formation of ethyl 2,3-epoxy-3-methylvalerate, which is then hydrolyzed to the free epoxy acid; in this, cleavage of the epoxide ring then occurs.



Our proposed modification of the method differs from the modification of the Claisen-Darzens method described by Sjolander and co-workers [2], in that we prepared the sodium ethoxide immediately before the synthesis (the synthesis of the epoxy ester was not successful when the sodium ethoxide was prepared beforehand by the method described by Adkins and Rainey [3]), and also in that we avoided the stage of the preparation and subsequent decomposition of the quinine salt of 2,3-dihydroxy-3-methylvaleric acid.



Infrared spectrum of sodium 2,3-dihydroxy-3-methylvalerate.

Procedure

A round-bottomed three-necked flask fitted with mercury-sealed mechanical stirrer and two reflux condensers, one carrying a dropping funnel, was charged with 23 g (1 g-atom) of sodium; 300 ml of purified dry ether was added, and then, slowly in drops, 57.5 ml (1 mole) of absolute ethanol was added. When the addition of the alcohol was complete, the mixture was boiled for 4 hr in a water bath. The sodium was first granulated by melting it in hot dry xylene and shaking; the xylene was then decanted, and the sodium was washed with dry ether.

After the preparation of sodium ethoxide in the same reaction flask, we added 50 ml of ether, and from a

dropping funnel we slowly added a mixture of 72 g (1 mole) of purified dry butanone and 110 g (0.9 mole) of ethyl chloroacetate [b.p. 141° (uncorr.)]. During the addition of the mixture the reaction vessel was cooled with a mixture of ice and salt (−15°). As the mixture was added there was a vigorous reaction: the ether boiled, and the reaction mixture gradually became orange. After 12 hr some ether–alcohol mixture was distilled from the reaction mixture, which was then heated in a boiling water bath for 3 hr.

The almost-clear orange liquid was poured onto 150 g of ice, and 15 ml of 50% H_2SO_4 was added carefully. The epoxy ester was extracted with two portions of ether, and the ether layer was washed repeatedly with water, then with sodium bicarbonate solution, and again with water. The reddish-brown ethereal extract was dried over a mixture of dry sodium and magnesium sulfates. Ether was distilled off, and the residual liquid was distilled at 2-3 mm from a Claisen flask through a vertical spiral condenser. We obtained two fractions: I, b.p. 30-60°, and II, b.p. 60-68°. Each fraction was refractionated with collection of subfractions boiling above 60°. These fractions were then fractionated further until fractions were obtained that did not give the Beilstein test for chlorine [4]. Such careful distillation was necessary for the complete removal of unchanged chloroacetic ester, the presence of which is extremely undesirable for biochemical investigations. We obtained 30 g (0.19 mole) of ethyl 2,3-epoxy-3-methylvalerate, which corresponds to a yield of 21%. In Sivolobov's apparatus [4], the ester had b.p. 199.5° (uncorr.). The literature gives: b.p. 199° [5]; b.p. 197-199° [6].

This ester (30 g) was hydrolyzed with 2.5 N NaOH (79.8 ml) for 15 min. The solution was extracted with ether. The aqueous layer was acidified with HCl. The upper layer then readily separated and was collected, and the lower aqueous layer was again extracted with ether. The ether extracts, together with the upper oily layer, were dried over $MgSO_4$. Ether was distilled off, and there remained a viscous syrup consisting of free 2,3-epoxy-3-methylvaleric acid. This epoxy acid was dissolved in 100 ml of water and, to open the epoxide ring, was refluxed for 4 hr. The solution was vacuum-evaporated down to a viscous liquid, b.p. 179° (uncorr.) in Sivolobov's apparatus. For free 2,3-dihydroxy-3-methylvaleric acid the literature [5] gives b.p. 185° (decomp.).

To prepare the sodium salt of this acid it was dissolved in a little water, neutralized with $NaHCO_3$, and vacuum-evaporated to dryness; the residue was then washed with absolute alcohol or dry acetone (to remove aldehyde formed as a result of the decarboxylation of this acid). Sodium 2,3-dihydroxy-3-methylvalerate had m.p. 114-115° (uncorr.).

In paper chromatography in various solvent systems sodium 2,3-dihydroxy-3-methylvalerate was developed both with an acid-base indicator [7] and with a periodate reagent for glycols (the paper was sprayed with saturated KIO_4 solution) [8]; and after drying of the chromatogram in a current of warm air it was sprinkled with a solution consisting of 0.5 g of benzidine base, 20 ml of glacial acetic acid, and 30 ml of ethanol [9]. Dihydroxy acids, like other glycols, develop as a white spot on a blue ground. The sodium salt obtained gave only one spot, i.e., was chromatographically pure. When a mixture of ether, benzene, and formic acid (70:30:11.2) was used as solvent [10], the spot had R_f = 0.71-0.73. The literature [10] gives R_f = 0.70.

Another method for the identification of the sodium salt obtained was provided by the almost-complete identity of its infrared spectrum (Fig. 1) with the published spectrum [11] of synthetic and natural free 2,3-dihydroxy-3-methylbutyric acid (dihydroxy analog of valine). The spectrum was interpreted according to Bellamy [12]. The minimum transmission at 1610 cm^{-1} belongs to the absorption of the COOMe group, the minima at 3290 and 3360 cm^{-1} are due to the presence of two different hydrogen bonds, and the minimum at 3590 cm^{-1} is determined by the presence of free OH groups in the molecule. Hence, in the crystalline state, sodium 2,3-dihydroxy-3-methylvalerate exists as the dimer, in which the two molecules are linked by a hydrogen bond, in addition to which there is also an internal hydrogen bond in the molecule.

The spectrum of the dry sodium salt (in compression with KBr) was determined with a UR-10 spectrograph by N. A. Gabelova (Institute of Biophysics, Academy of Sciences of the USSR), and L. G. Erokhina (A. N. Bakh Institute of Biochemistry, Academy of Sciences of the USSR). In the interpretation of the spectrum much assistance was given by A. V. Karyakin (V. I. Vernadskii Institute of Geochemistry and Analytical Chemistry, Academy of Sciences of the USSR). To all these workers we express our deep gratitude.

SUMMARY

The synthesis of sodium 2,3-dihydroxy-3-methylvalerate (the 2,3-dihydroxy analog of isoleucine) is described.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
