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The Reaction of Djenkolic Acid and Aqueous Calcium Hydroxide. The Isolation and Identification of Racemic Thiazolidine-4-carboxylic Acid

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One of the materials produced by the treatment of djenkolic acid at room temperature with aqueous calcium hydroxide has been identified as thiazolidine-4-carboxylic acid. The isolation, purification and comparison of this material with the synthetic compound have been described. The identity of this material has been further confirmed by comparison of its infrared and ultraviolet absorption spectra with those of the synthetic material.

Introduction

Djenkolic acid (I) $CH_2(SCH_2CH(NH_2)COOH)_2$ (Chart I) is a naturally occurring sulfur-containing amino acid which was first detected in the urine of natives of Java who included the djenkol bean in their diet. It was separated from djenkol beans in 1935 by van Veen and Hyman,[1] who extracted the djenkol beans with dilute barium hydroxide at 30° and isolated the acid from the reaction mixture. The acid was found to be stable to concentrated hydrochloric acid at 100°, but hydrolysis with concentrated sulfuric acid gave cystine and formaldehyde. Saturated barium hydroxide gave no sulfites or sulfates with djenkolic acid. A dibenzoyl derivative and a dihydantoin were prepared. The synthesis of djenkolic acid was carried out by du Vigneaud and Patterson,[2] who treated cystine in liquid ammonia with methylene chloride. Armstrong and du Vigneaud[3] later synthesized djenkolic acid from 1 mole of formaldehyde and 2 moles of cysteine in strong hydrochloric acid solution. Middlebrook and Phillips[4] obtained djenkolic acid by adding formaldehyde to a wool cystine subfraction. Evidence that djenkolic acid occurs in a free state was obtained by van Veen and Latuasan,[5] who separated djenkolic acid from djenkol beans by a process of dialysis, extraction and chromatographic absorption, while Ågren and Eklund[6] obtained djenkolic acid from a hydrolyzate of a peptide in calf plasma. They also found traces in muscle tissue.

In the present work, djenkolic acid was treated with aqueous calcium hydroxide at room temperature for 2 months in the same manner as was used in earlier work in these Laboratories for the similar treatment of cystine.[7] The calcium salt II was obtained by drying down the filtered reaction mixture at room temperature. Esterification of this salt with absolute ethanol and dry hydrogen chloride, followed by neutralization with potassium carbonate and distillation *in vacuo*, gave the liquid ester III in 30% yield, which, upon treatment with dry hydrogen chloride, gave the solid ester hydrochloride IV. Elemental analysis indicated that this was the ethyl ester of thiazolidine-4-carboxylic acid (VII) and not the diethyl ester of 2-methylthiazolidine-2,4-dicarboxylic acid (IX) as had been obtained from cystine treated with aqueous calcium hydroxide.[7] The analysis also indicated that the material was not the diethyl ester of djenkolic acid (VIII). The ester VII was prepared from synthetic thiazolidine-4-carboxylic acid (VI), which, in turn, had been prepared from L-cysteine (V), and formaldehyde by the method of Ratner and Clarke.[8]

It was found that the hydrochloride[9] of the synthetic ester VII melted some 20° higher than the hydrochloride of the ester IV obtained from djenkolic acid treated with aqueous calcium hydroxide. Ultraviolet absorption spectra of the two compounds were identical but showed no characteristic absorption. Infrared absorption spectra also indicated that the two compounds VII and IV were apparently identical; only the spectra (Fig. 1) of the synthetic compound VII is given since the two spectra are nearly indistinguishable. Infrared absorption spectra were obtained on a Baird double-beam spectrophotometer using rock-salt optics. The samples were prepared as pressed plates in potassium bromide.

Since a racemized thiazolidine was isolated from cystine which had been treated with aqueous calcium hydroxide,[7] it seemed quite plausible to expect the same phenomena in the case of djenkolic acid. This proved to be the case, as the synthetic ester hydrochloride VII was found to have a specific rotation $[\alpha]_D^{28.4}$ of $-79.0°$ in alcohol (c 0.01 g./cc.), while the ester hydrochloride IV obtained from djenkolic acid was found to be optically inactive. When the synthetic ester hydrochloride VII was treated with a catalytic amount of potassium hydroxide in ethanol by the procedure of McKenzie and Wren,[10] the low-melting ester hydrochloride IV was obtained. When untreated djenkolic acid (I), was esterified in the same manner as was II, a solid ester hydrochloride VIII was obtained which was not similar to either IV or VII.

Experimental

Aqueous Calcium Hydroxide Treatment of Djenkolic Acid.—Thirty grams of djenkolic acid[3] and 30 g. of calcium hydroxide were mixed with 750 ml. of distilled water and left at 25° for 53 days. The reaction mixture was then filtered and 375 ml. of the clear filtrate was evaporated to dryness *in vacuo* with gentle warming on a steam-bath; 200 ml. of absolute ethanol was added to the dry residue,

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[1] A. G. van Veen and A. J. Hyman, *Geneesk. Tijdschr. Nederl. Indie*, **73**, 991 (1933); *Rec. trav. chim.*, **54**, 493 (1935).

[2] V. du Vigneaud and W. I. Patterson, *J. Biol. Chem.*, **114**, 533 (1936).

[3] M. D. Armstrong and V. du Vigneaud, *ibid.*, **114**, 373 (1947).

[4] W. B. Middlebrook and H. P. Phillips, *Biochem. J.*, **41**, 218 (1947).

[5] A. G. van Veen and H. E. Latuasan, *Chronica Naturae*, **105**, 288 (1949); *C. A.*, **44**, 4548 (1950).

[6] G. Ågren and S. Eklund, *Acta Chem. Scand.*, **6**, 1129 (1952).

[7] J. R. Dann, G. L. Oliver and J. W. Gates, Jr., THIS JOURNAL, **79**, 1644 (1957).

[8] S. Ratner and H. T. Clarke, *ibid.*, **59**, 200 (1937).

[9] H. T. Clarke, J. R. Johnson and R. Robinson, editors, "The Chemistry of Penicillin," Princeton University Press, Princeton, N. J., 1949, p. 937.

[10] A. McKenzie and H. Wren, *J. Chem. Soc.*, **115**, 602 (1919).

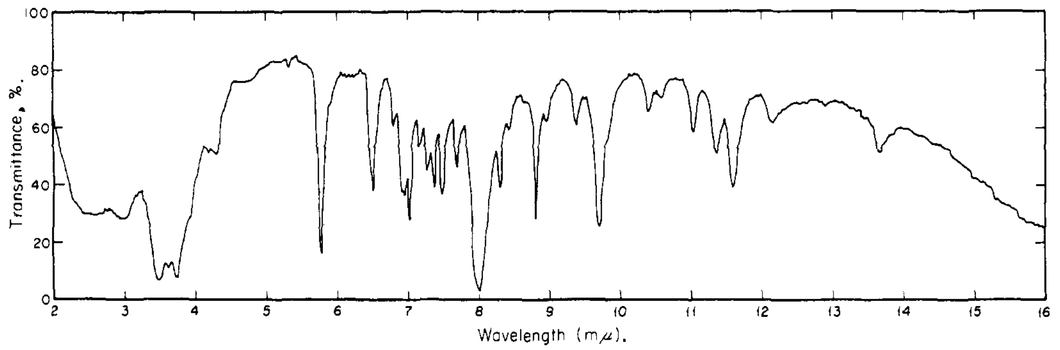
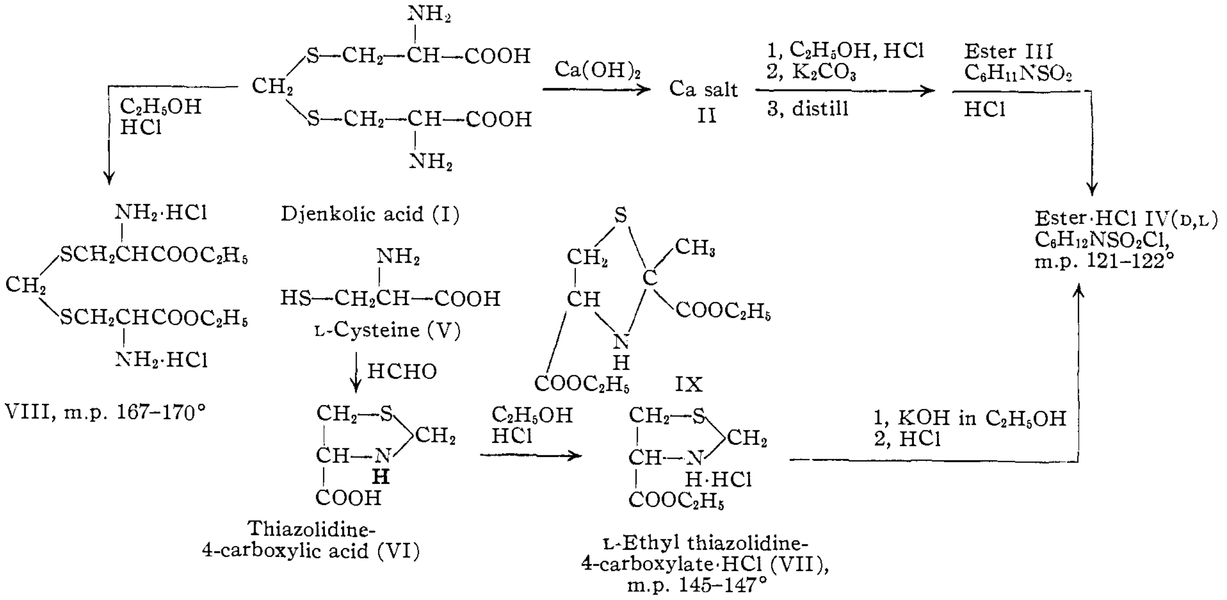


Fig. 1.—Infrared spectra of synthetic ethyl thiazolidine-4-carboxylate hydrochloride (VII).

CHART I



and dry hydrogen chloride was bubbled into the alcohol for 5 minutes. After refluxing for 15 minutes, the reaction mixture was left overnight at room temperature and the alcohol was then distilled off. The residue was washed with ether and then made alkaline with 15 g. of potassium carbonate in 150 ml. of water. This was extracted with ether, and the ether was distilled off, after being dried over anhydrous magnesium sulfate, to give 6.6 g. of dark oil. This oil was distilled *in vacuo* with a Cenco HyVac pump and a 5-cm. Vigreux column to give 3.15 g. (32.2%) of distillate boiling at 80–84° at 3 mm. When this distillate was treated with dry hydrogen chloride in dry ether, a crude precipitate was obtained melting at 96–101°. Several recrystallizations from absolute ethanol gave a product with a melting point of 121–122°.

Anal. Calcd. for the hydrochloride of ethyl thiazolidine-4-carboxylate, $C_6H_{12}NSO_2Cl$: C, 36.5; H, 6.08. Found: C, 36.5; H, 6.3; C, 36.2; H, 6.1.

Thiazolidine-4-carboxylic Acid.—This was prepared by the method of Ratner and Clarke[8] from Eastman Kodak Co. cysteine hydrochloride and formaldehyde, m.p. 205° dec.

Ethyl Thiazolidine-4-carboxylate Hydrochloride.[9]—This was prepared from thiazolidine-4-carboxylic acid by the method used by Ratner and Clarke,[8] for the preparation of the methyl ester, m.p. 145–147°, $[\alpha]^{28.9}D$ 79° in alcohol (*c* 0.01 g./cc.).

Ethyl Thiazolidine-4-carboxylate.—This was prepared from the ethyl thiazolidine-4-carboxylate hydrochloride by the method used by Ratner and Clarke[8] for the preparation of the methyl ester, b.p. 90–91° (3 mm.).

Anal. Calcd. for $C_6H_{11}NSO_2$: C, 44.65; H, 6.83; N, 8.7. Found: C, 44.5; H, 7.2; N, 8.4.

Racemization[10] **of Ethyl Thiazolidine-4-carboxylate.**—Ethyl thiazolidine-4-carboxylate, 0.322 g. (0.002 mole), was left overnight at 25° in 20 ml. of absolute ethanol containing 0.0561 g. (0.001 mole) of potassium hydroxide. The reaction mixture was then filtered and the alcohol distilled off *in vacuo* to leave a clear yellow oil. This was extracted with two 15-ml. portions of ether which were combined. Upon bubbling dry hydrogen chloride into the ether, a white precipitate melting at 118–120° was formed (0.08 g.). The mixed melting point with the hydrochloride of the ester IV obtained from treated djenkolic acid was not depressed.

Preparation of the Hydrochloride of Diethyl Djenkolate.—Dry hydrogen chloride gas was passed for 5 minutes into a solution of 3.75 g. of djenkolic acid in 100 ml. of absolute ethanol. The reaction mixture was refluxed for 6 hr. and then reduced to a volume of 20 ml. *in vacuo*. Crude crystals were obtained (3.5 g.), m.p. 155–165°. Recrystallization from absolute alcohol-ether gave a m.p. of 170–172°.

Anal. Calcd. for $C_{11}H_{24}N_2S_2O_4Cl_2$: C, 34.5; H, 6.26; N, 7.32; S, 16.7. Found: C, 33.7; H, 6.1; N, 7.4; S, 17.0.

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