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106. Tadashi Fujita, Akira Ayada, and Yoshitaka Mushika : Studies on Carnitine. I. Hydrolysis of Carnitine-nitrile Chloride with Concentrated Hydrochloric Acid.

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In the filtrate of extract of yeast and liver, Fraenkel, *et al.*,[1] first discovered a growth factor for larvae of Tenebrio-molitor. They named the growth factor "Vitamin B_T" and described its properties. Carter, *et al.*[2] succeeded in isolating a crystalline substance possessing Vitamin B_T activity and in the following year they identified this substance as carnitine (4-trimethyl-3-hydroxybutyrobetaine) (I).

The synthesis of (I) had been performed by Tomita,[3] Bergman,[4] Carter,[5] Engeland,[6] and Strack,[7] of whom the two latters hydrolyzed carnitinenitrile chloride [(3-cyano-2-hydroxypropyl) trimethylammonium chloride] (II) to yield carnitine hydrochloride [(3-carboxy-2-hydroxypropyl)trimethylammonium chloride] (III) and isolated the latter via the auric chloride salt (Engeland) or via reineckate (Strack), thus these methods of preparation were rendered unpractical.

Recently Belgian patent No. 519, 286 and Dechamps[8] described a crystalline compound

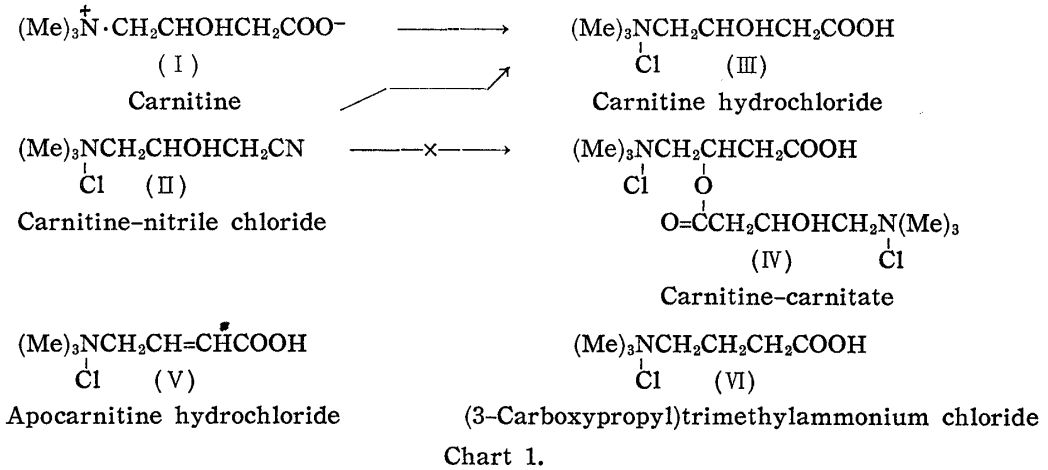

Chart 1.

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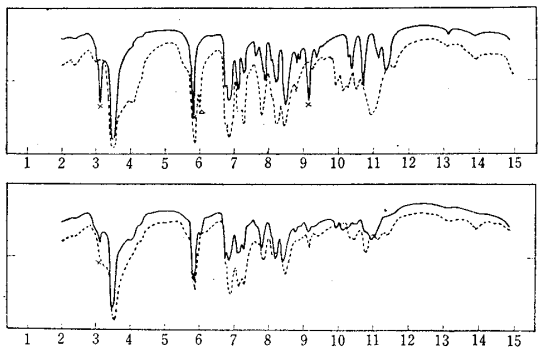
1) G. Fraenkel, M. Blewett : Biochem. J., **41**, 469 (1947); Nature, **161**, 981 (1948).
2) H. E. Carter, D. K. Bhattacharyya, K. R. Weldmann, G. Fraenkel : Federation Proc., **10**, 170 (1953); Arch. Biochem. Biophysic., **38**, 405 (1952); **35**, 241 (1952).
3) M. Tomita : Z. physiol. Chem., **124**, 253 (1923).
4) M. Bergman, F. Rodt : Ber., **54**, 1648 (1921).
5) H. E. Carter, D. K. Bhattacharyya : J. Am. Chem. Soc., **75**, 2503 (1953).
6) R. Engeland : Ber., **43**, 2705 (1910).
7) E. Strack, H. Röhnet, J. Lorenz : *Ibid.*, **86**, 525 (1953).
8) M. G. Dechamps, N. P. Buo-Hoi, H. LeBihan, F. Binon : Compt. rend., **238**, 826 (1954).

Carnitine-nitrile chloride : 45 g.
Conc. HCl : 75 cc.
↓ at 120～130°, 10 hr.
NH_4Cl, 11.3 g. ← filtrate
↓ evapd. added anhyd. MeOH and Me_2CO
Crystal A, 31 g. ← filtrate
↓ evapd. added anhyd. MeOH and anhyd. Me_2CO
white crystal, 0.9 g. ← filtrate
↓ recrystd. from anhyd. EtOH
white prisms 0.5 g., m.p. 202～203° (decomp.)
Apocarnitine hydrochloride
filtrate → evapd. added anhyd. EtOH and anhyd. Me_2CO
white crystal, 0.7 g. ← filtrate
↓ recrystd. from anhyd. EtOH
white prisms 0.3 g., m.p. 202～203° (decomp.)
Apocarnitine hydrochloride
filtrate → evapd.
syrups, 11 g.

Chart 2.

Crystal A : 25 g.
↓ recrystd. from 95% EtOH
white prisms 7.2 g., m.p. 195～200° (decomp.) ← filtrate
↓ recrystd. from 95% EtOH
white prisms 2.6 g., m.p. 202～203° (decomp.) ← filtrate
Apocarnitine hydrochloride
filtrate → evapd. recrystd. from anhyd. EtOH
white prisms 9.4 g., m.p. 155～190° ← filtrate
filtrate → evapd. recrystd. from 95% EtOH
white prisms 2.0 g., m.p. 202～203° (decomp.) ← filtrate
Apocarnitine hydrochloride
filtrate → recrystd. from 95% EtOH
white prisms 4.6 g., m.p. 180～185° ← filtrate
↓ recrystd. from 95% EtOH
white prisms, 1.5 g. m.p. 195～196°
Carnitine hydrochloride
filtrate

Chart 3.



—— Carnitine hydrochloride (III)
------ Apocarnitine hydrochloride (V)

—— Hydrolyzed product : crystal A
------ Reduction product of crystal A

Fig. 1. Infrared Spectra

of m.p. 198~200° called carnitine-carnitate (IV), which is the intermolecular ester of two molecules of (III), as a stable derivative of (I). They claimed to have prepared this ester by heating (II) with conc. hydrochloric acid in an autoclave at 130°, which procedure was also claimed to be useful.

Being interested in carnitine, the hydrolysis of (II) was studied as a means of preparation of carnitine. Dechamps' method[8] was also traced, when it was found that the crystalline substance obtained in experiments performed according to Dechamps was a mixture of (III) and apocarnitine hydrochloride [(3-carboxy-2-propenyl)trimethylammonium chloride] (V), but the presence of a crystalline compound corresponding to (IV) had not been traced in the reaction mixture. Moreover by modifying the hydrolysis temperature, (III) was prepared directly from (II).

As shown in Chart 2, a mixture of (II) and conc. hydrochloric acid was heated in an autoclave at 120~130° for 10 hours with stirring and the product was allowed to stand overnight at room temperature. The filtrate from ammonium chloride separated was concentrated on a steam bath to dryness *in vacuo* and the resultant residue was dried over phosphoric anhydride. The solid thus obtained was treated with anhydrous methanol-anhydrous acetone and the crystals separated (A) were collected on a filter. By comparing its infrared spectrum ($\nu_{C=O}$ 5.80, 5.84, $\nu_{C=C}$ 6.0, ν_{OH} 3.10, ν_{-C-O-} 9.15 μ: see Fig. 1) with that of pure carnitine hydrochloride (III) and apocarnitine hydrochloride (V), this compound (A) was surmised to be a mixture of the two latter substances.

When reduced catalytically over Adams platinum-catalyst in 95% ethanol solution, (A) absorbed 1.2 molor equivalents of hydrogen based on (V). As shown in Fig. 1 $\nu_{C=O}$ absorption originally present in (A) is no more discernible in the reduction product.

Engeland[9] also observed an excessive absorption of hydrogen when apocarnitine hydrochloride (V) was reduced catalytically over palladium-catalyst, which phenomenon has its origin in the over-reduction. Thus the presence of trimethylamine and butyric acid beside (3-carboxypropyl)trimethylammonium chloride (VI) was recognized in the reduction product.

This reduction experiment also proceeded similarly and a distinct oder of butyric acid was evident in the reduction solution, which probably contained also trimethylamine, (III), (VI), rendering the presence of (V) in (A) probable.

The filtrate from (A) was concentrated *in vacuo* and the resultant residue was crystallized first from anhydrous methanol-anhydrous acetone and then from anhydrous ethanol-anhydrous acetone yielding 1.6 g. of a crystalline substance. Judging from its infrared spectrum this was presumed to be crude (V) and further purification from anhydrous ethanol furnished colorless prisms, m.p. 202~203° (decomp.), which exhibited an identical infrared spectrum with the authentic apocarnitine hydrochloride (V), prepared by dehydrating (III) by conc. sulfuric acid according to the procedure of Engeland.[9] The result of the mixed melting point test was also satisfactory.

Approximately 11 g. of a syrupy substance, which was left uninvestigated by Dechamps, were recovered from the mother liquor of (A). From inspection of its infrared spectrum this is supposed to be a mixture of (III) and (V) and their methyl esters. The latter were probably formed when the substance containing (A) and some residual hydrochloric acid was heated with anhydrous methanol with purpose of isolating (A).

As afore-mentioned, the presence of (V) in (A) now became fairly certain. Thus as is shown in Chart 3, 25 g. of (A) was fractionally recrystallized from 95% ethanol and anhydrous ethanol to furnish 4.6 g. of pure (V) and 1.5 g. of pure (III) were identified through

9) R. Engeland: Ber., 54, 2208 (1921).

elemental analysis, mixed melting point test and infrared spectrum. Besides them some of a mixture of these two compounds was also obtained.

We further investigated the behavior of (III) toward conc. hydrochloric acid the same conditions of the hydrolysis of (II) and found, as was expected, that most of the starting material was converted to (V).

In conclusion it became questionable that Dechamps, *et al.* obtained carnitine-carnitate (IV) in their hands as the hydrolysis product of (II), since the latter, when heated with conc. hydrochloric acid at 130°, yielded mainly (V) containing a certain amount of (III) in the present work.

By carrying out the hydrolysis of (II) at boiling water bath temperature the formation of (V) was practically suppressed to give (III) in a satisfactory yield, the formation of (IV) was altogether not observed.

Experimental

Hydrolysis of (3-Cyano-2-hydroxypropyl)trimethylammonium Chloride (II) with Concentrated Hydrochloric Acid——A mixture of (II) (45 g.) and conc. HCl (75 cc.) was heated with stirring for 10 hr. in autoclave at 120~130° and allowed to stand at room temperature overnight. The separated crystals were filtered, washed with 95% EtOH (50 cc.) and dried. The crude NH_4Cl was obtained (11.3 g., 83.8%).

The combined mother liquors were evaporated to dryness under reduced pressure on a steam bath and dried over P_2O_5. The residue was dissolved in anhyd. MeOH (50 cc.) by warming and the solution after cooling to room temperature was diluted with anhyd. Me_2CO (500 cc.). The separated crystals named (A) (31 g.) were obtained. (A) decomposed at 180~190° and showed IR λ_{max}^{Nujol} μ: 3.10, 5.80, 6.0, 9.15, was described in next part.

The mother liquor from (A) was treated as shown in chart 2. Then, the white prism crystal (0.8 g.) was obtained. m.p. 202~203° (decomp.), IR λ_{max}^{Nujol} μ: 5.84, 6.01.

This compound was identified as (V) by the mixed melting point test and infrared spectrum of authentic sample.[9]

The combined filtrate of (V) was concentrated *in vacuo* to yield 11 g. of syrupy substance (IR λ_{max}^{Nujol} μ: 3.10, 5.75, 5.80, 6.0, 9.19).

Examination of Crystal (A)

i) Catalytic reduction: A solution of (A) (3.6 g.) in 95% EtOH (100 cc.) was hydrogenated at atmospheric pressure and 20° using Adams PtO_2 catalyst (100 mg.). 555 cc. of hydrogen (calculated hydrogen volumes based on (V) are 448 cc.) was absorbed in 80 min.

After filtering the catalyst, the filtrate was evaporated to dryness under reduced pressure and the residue was recrystallized twice from 95% EtOH. Yield, 0.2 g., m.p. 195~196°.

It was identified as (III) by the mixed melting point and infrared spectrum.

The mother-liquor from (III) was concentrated *in vacuo* on the steam bath. The residue was dissolved in a minimum anhyd. MeOH and diluted with anhyd. Me_2CO.

After cooling in ice-box overnight, the crystals were collected to yield 0.6 g., m.p. 147~190°, IR λ_{max}^{Nujol} μ: 5.84, 9.14. This crystal could not be separated to the pure substance but was assumed the mixture of (III) and (VI).

The filtrate from the above crystal was evaporated to dryness again. The residue was treated with anhyd. Me_2CO. Trimethylamine hydrochloride was obtained as a white prisms, m.p. 260~265° (decomp.), picrate, m.p. 216°.

ii) Fractional crystallization: The fractional crystallization of (A) (25 g.) was carried as shown in Chart 3. (V), 4.6 g., m.p. 202~203° (decomp.), IR λ_{max}^{Nujol} μ: 5.84, 6.0 and (III), 1.5 g., m.p. 195~196°, IR λ_{max}^{Nujol} μ: 3.1, 5.80, 9.15 were obtained as white prisms. The mother-liquor from (III) and (V) was evaporated to dryness. The residue was recrystallized from EtOH to yield additional (III) and (V).

Heating of the (III) with Concentrated Hydrochloric Acid (Formation of (V))——A mixture of (III) (19.8 g.) and conc. HCl (20 g.) was heated in an autoclave at 120~130° for 10 hr. and allowed to stand overnight at room temperature. The reaction mixture was treated with anhyd. Me_2CO (100 cc.) and the separated crystals were filtered. The filtered crystals (m.p. 180~190°, 17.5 g.) were recrystallized twice from 95% EtOH to yield 7.6 g., white prisms, m.p. 202~203° (decomp.). It was identified with (V) by the mixed melting point test and infrared spectrum.

The mother-liquor from (V) was evaporated to dryness *in vacuo*. The residual crystals showed in IR λ_{max}^{Nujol} μ: 3.10, 5.80, 5.84, 6.0, 9.15, so it was considered as the mixture of (III) and (V) but the separation of the mixture was very difficult.

Carnitine hydrochloride [(3-Carboxy-2-hydroxypropyl)trimethylammonium Chloride] (III)——A mixture of (II) (150 g.) and conc. HCl (171 g.) was heated with stirring for 4 hr. in a boiling water-bath and allowed to stand overnight at room temperature. The separated crystals were filtered off and washed with MeOH (300 cc.) to yield about 43.8 g. of crude NH_4Cl.

The mother-liquor from NH_4Cl was concentrated under reduced pressure on a steam bath. The residue was dissolved in water and the solution was decolorlized with charcoal and evaporated to dryness *in vacuo*.

The residue was treated with EtOH (200 cc.). The separated crude (III) was filtered.

The mother-liquor was again evaporated and treated with EtOH. In repeating this procedures, additional crude (III) was obtained. Total yield, 144.5 g., m.p. 187~191°, 87%.

Crude (III) was recrystallized from 95% EtOH or MeOH to yield (III) white prisms, m.p. 195~196°, 85%. *Anal.* Calcd. for $C_7H_{16}O_3NCl$: C, 42.51; H, 8.16; N, 7.09. Found : C, 42.76; H, 8.37; N, 7.05.

Salts of Carnitine

Sulfamate : White needles (from MeOH), m.p. 147~148°. *Anal.* Calcd. for $C_7H_{18}O_6N_2S$: C, 32.55; H, 7.02; N, 10.85. Found : C, 33.05; H, 6.67; N, 11.27.

Cyclohexane-sulfamate : White prisms (from EtOH-Me_2CO), m.p. 121~122°. *Anal.* Calcd. for $C_{13}H_{28}O_6N_2S$: C, 45.86; H, 8.29; N, 8.23. Found : C, 46.00; H, 8.36; N, 8.63.

Sulfate : White prisms (from MeOH), m.p. 156~157°. *Anal.* Calcd. for $C_{14}H_{32}O_{10}N_2S_2$: C, 39.99; H, 7.67; N, 6.66. Found : C, 39.89; H, 8.15; N, 6.87.

Nitrate : White prisms (from EtOH), m.p. 79~81°. *Anal.* Calcd. for $C_7H_{16}O_6N_2$: C, 37.49; H, 7,19; N, 12.50. Found : C, 36.99; H, 7.36; N, 12.56.

Orotate : White powder (from MeOH-EtOH), m.p. 191°. *Anal.* Calcd. for $C_{12}H_{19}O_7N_3$: C, 45.42; H, 6.04; N, 13.24. Found : C, 45.25; H, 6.32; N, 13.49.

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Summary

It became questionable that Dechamps, *et al.*[8] obtained carnitine-carnitate (IV) in their hands as the hydrolysis product of carnitine-nitrile chloride (II), since in our hands the latter, when heated with conc. hydrochloric acid at 130°, yielded mainly apocarnitine hydrochloride (V) containing a certain amount of carnitine hydrochloride (III). By carrying out the hydrolysis of (II) at boiling water-bath temperature the formation of (V) was practically suppressed to give (III) in a satisfactory yield, the formation of (IV) was altogether not observed.

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