An Unsaturated Cyclic Alcohol in the Nonsaponifiable Matter of the Liver Oil of the Crab, Paralithodes Camtschatica

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By Suketaka ITO[*1] and Yoshiyuki TOYAMA[*2]

The presence of an unsaturated alcohol with the formula $C_{10}H_{18}O_2$ in the nonsaponifiable matter of the liver oil of the crab, *Palalithodes Camtschatica*, was reported forty years ago by Tsujimoto.[1] This alcohol, named kanyl alcohol by him, was shown to be an unsaturated monohydric alcohol, whereas the nature of the other oxygen atom was not decided. It was postulated as having a branched chain of carbon atoms or a cyclic structure from the fact that both this alcohol and its hydrogenation product had low freezing points. The hydroxyl group of this alcohol was inferred to be located in a position which is unfavorable for urethan formation, since neither phenyl urethan nor α-naphthyl urethan of a crystalline form could be obtained from this alcohol. Later, Nakamiya[2] reinvestigated a fraction corresponding to the kanyl alcohol of Tsujimoto and concluded that kanyl alcohol was not a uniform substance but a mixture of different components.

In this study, the liquid fraction was separated from the nonsaponifiable matter of the liver oil of the crab, *Paralithodes Camtschatica*. The acetate of this fraction was fractionally distilled to give an acetate fraction which accords with Tsujimoto's kanyl alcohol in its refractive index, saponification value and iodine value. However, when this fraction was subjected to chromatography on silica gel, a main fraction and a by-fraction were collected in the ratio of approximately 2 : 1. The gas-liquid chromatography of these two fractions indicated that while the by-fraction contained at least several components, the main fraction consisted almost exclusively of a uniform substance. When the main fraction was studied further, the conclusion was reached that the free alcohol, $C_{12}H_{20}O_2$, corresponding to this acetate fraction is α-octa-2, 5-dienyl-β-hydroxytetrahydrofuran.

Experimental

The Properties of Liver Oil.—A 37.35 kg. batch of livers separated from crabs, *Paralithodes Camtschatica*, caught in northern waters was supplied by the Nihon Suisan K. K. It was heated on a water bath to remove the bulk of the water; the concentrated material, 11 kg., was extracted with ether, yielding 2.033 g. of oil. The oil was completely soluble in acetone. It was a dark brown liquid with a peculiar odor and had the following characteristics: d_4^{20} 0.9280, n_D^{20} 1.4796, A. V. 89.4, S. V. 160.4, I. V. (Wijs) 174.1[*3] and nonsaponifiable matter 11.0 %.

The Separation of the Liquid Fraction of Nonsaponifiable Matter and the Fractional Distillation of Its Acetate. — A crude nonsaponifiable matter was separated from the oil by saponification with potassium hydroxide in ethanol, followed by the extraction of the soap solution with ether in the usual way. A 202 g. portion of the crude nonsaponifiable matter was dissolved in 600 ml. of hot methanol, and the solution was cooled with ice to separate 53 g. of a crystalline solid which consisted chiefly of cholesterol. The solid was filtered, and the material recovered from the filtrate was dissolved in 440 ml. of hot acetone. When the acetone solution was cooled, 14.5 g. of a crystalline solid was separated and filtered. This solid consisted chiefly of batyl alcohol, together

TABLE I. THE FRACTIONAL DISTILLATION OF THE ACETATE OF LIQUID NONSAPONIFIABLE MATTER

Fraction	B. p. °C/3.5 mmHg	Yield g.	n_D^{23}	S. V.	I. V.
1	~130	6.0	1.4599	259.8	124.6
2	130~140	12.3	1.4642	266.5	142.1
3	140~150	2.2	1.4645	277.9	129.5
4	150~160	1.3	1.4644	274.4	116.7
5	160~170	1.9	1.4634	275.2	96.1
6	170~180	1.8	1.4627	268.4	92.1
7	180~190	3.3	1.4609	259.5	78.9
8	190~200	2.5	1.4594	245.8	67.3
9	200~210	6.0	1.4586	228.2	57.1
10	210~219	16.0	1.4582	225.6	50.3
11	219~220	6.4	1.4590	224.4	49.8
12	220~223	15.1	1.4605	218.1	56.1
13	223	6.7	1.4663	211.5	71.7
14	Residue	13.0	—	—	—

[*1] Present address: Department of Industrial Chemistry, Faculty of Engineering, Shinshu University, Nagano-shi.

[*2] Present address: Department of Applied Chemistry, Faculty of Engineering, Toyo University, Kawagoe, Saitama-ken.

1) M. Tsujimoto, *Repts. Govern. Chem. Ind. Res. Inst., Tokyo*, **23** [3], 25 (1928); **24** [4], 9 (1929).

2) Z. Nakamiya, *Bull. Inst. Phys. Chem. Res.*, **19**, 790 (1940).

[*3] The abbreviations A. V., S. V. and I. V. are used for acid value, saponification value and iodine value respectively.

with a small amount of cholesterol. From the acetone filtrate a liquid fraction of nonsaponifiable matter was recovered (n_{D}^{22} 1.4898, A. V. 8.4 and I. V. 142.8). It was acetylated by refluxing it with acetic anhydride to give an acetate of S. V. 206.9 and I. V. 100.7. A 145 g. portion of this acetate was distilled to give a distillate of b. p. 110~223°C/2 mmHg, S. V. 231.2 and I. V. 84.3. The fractional distillation of this distillate (94.5 g.) gave the results shown in Table I.

Fraction 1 and fractions 3—6 in Table I were refractionated. A fraction approximating fraction 2 was separately collected and united to fraction 2. This united fraction was fractionated further to give, eventually, 8.7 g. of a fraction with b. p. 130~135°C/2 mmHg, n_{D}^{22} 1.4624, S. V. 265.0 and I. V. 143.9. These values approximate those for kanyl acetate reported by Tsujimoto:[1] n_{D}^{20} 1.4623, S. V. 251 and I. V. 145.

The Chromatography of the Acetate Fraction (B. p. 130~135°C/2 mmHg).—This fraction (8.5 g.) was chromatographed on silica gel and eluted with hexane and then with hexane-ether (100 : 0.5, 100 : 1 and 100 : 2). Each 100 ml. of the eluate was separately collected, giving a total of 60 fractions. The chromatogram is shown in Fig. 1. The eluate fractions 5—14 were united, and 2.6 g. of united material were rechromatographed on silica gel. After the earlier and later eluate fractions had been removed, the intermediate fractions, 2.05 g. in total (n_{D}^{20} 1.4703~1.4712) were combined into a fraction A of S. V. 235.5 and I. V. 196.5 (calculated for $C_{12}H_{19}O\cdot OCOCH_3$: S. V. 235.4 and I. V. 213.0). Fraction A was analyzed by gas-liquid chromatography at 210°C, using a 225 cm × 6 mm. column of diethylene glycol succinate polyester on Celite 545. The chromatogram indicated that this fraction contained a uniform substance almost exclusively. The infrared spectra of this fraction and the free alcohol obtained by saponification are shown in Fig. 2.

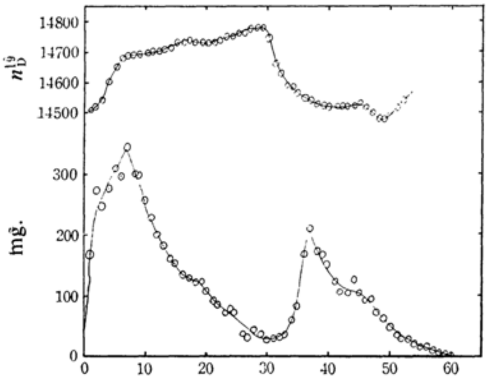

Fig. 1. Elution chromatogram of the acetate fraction, b. p. 130~135°C/2 mmHg.

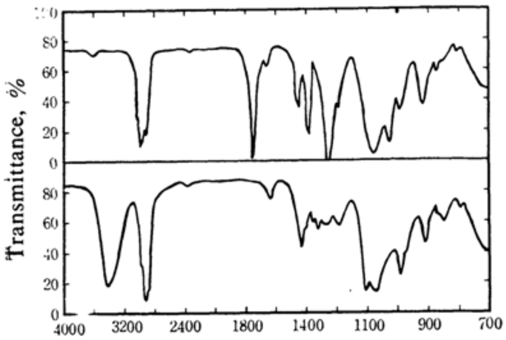

Fig. 2. Infrared spectra of the fraction A (upper) and the free alcohol (lower). Film on NaCl flats.

The eluate fractions 36—46 in Fig. 1 were united, and the united material, 1.55 g., was chromatographed on silica gel to give 0.96 g. of a fraction B having S. V. 322.7 and I. V. 107.6, but this fraction was found by gas-liquid chromatography to be a complicated mixture of different components.

Alkali-Isomerization and Ozonolysis of Fraction A. — Fraction A, after isomerization with a 6.6% solution of potassium hydroxide in ethylene glycol at 180°C for 25 min., exhibited the characteristic absorption of conjugated dienes, $k_{233}=37.6$.

A 100 mg. portion of fraction A in 10 ml. of chloroform was ozonized at −15°C. Then 10 ml. of water was added to the solution, and the mixture was gradually heated from 20°C to 90°C while a current of hydrogen was being passed through the mixture. The volatile substances formed were carried off by the hydrogen current and passed successively through a trap containing a solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid and a trap containing a solution of barium hydroxide. A considerable amount of precipitates of barium carbonate was formed in the second trap. The precipitates of 2,4-dinitrophenylhydrazone (DNPH) formed in the first trap were filtered. The DNPHs in the filtrate were recovered by extraction with benzene, followed by washing of the benzene solution with 2 N hydrochloric acid and water, and finally by the distilling off of the benzene. The total yield of crude DNPHs was 294 mg. Crude DNPHs were chromatographed on silica gel. Two eluate fractions were collected using hexane and hexane-ether as the eluants. The first eluate fraction gave 90 mg. of a DNPH which was then purified by rechromatography on silica gel, followed by recrystallization from hexane. The purified substance was in the form of orange yellow needles of m. p. 155.5~156.5°C (lit.[3] 155°C for propionaldehyde DNPH).

Found: N, 23.40. Calcd. for $C_9H_{10}N_4O_4$: N, 23.50%.

The second eluate fraction gave 78 mg. of a DNPH which was purified by rechromatography on silica gel and recrystallization from hexane to give, eventually, yellow needles of m. p. 165~165.5°C (lit.[4] 168.5°C for acetaldehyde DNPH). No depression of the melting point was observed on admixture with an authentic specimen of acetaldehyde DNPH.

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[3] C. F. H. Allen, *J. Am. Chem. Soc.*, **52**, 2955 (1930).

[4] W. M. D. Bryant, ibid., **60**, 2814 (1938).

A Saturated Alcohol from the Hydrogenation Product of Fraction A.—Fraction A was hydrogenated in ethanol using platinum black as the catalyst. The hydrogenation product was saponified to give a crude saturated alcohol which had n_D^{17} 1.4581 and I. V. 6.2. It was a pale yellow liquid with a solidifying point below −15°C, which was negative to the iodoform reaction. This material was used as a saturated alcohol in the experiments described below.

Phenyl Urethan. — A solution of 50 mg. of the saturated alcohol in 3 ml. of benzene, which had been dried over metallic sodium, was refluxed with 50 mg. of phenyl isocyanate for 30 min. When the solvent and the excess of the reagent were distilled off, the reaction product, phenyl urethan, was obtained in a yield of 80 mg. It did not solidify on standing at room temperature. Only after repeated heating and cooling did it begin to solidify. After repeated recrystallizations from hexane, fibrous crystals of m. p. 57.5~58.5°C were obtained.

Found: C, 71.22; H, 9.08; N, 4.84. Calcd. for $C_{19}H_{29}O_3N$: C, 71.44; H, 9.15; N, 4.39%.

The Oxidation of the Saturated Alcohol with Chromic Acid. — A solution of 100 mg. of chromic anhydride in 5 ml. of 90% acetic acid was gradually added to a solution of 180 mg. of the saturated alcohol in 10 ml. of glacial acetic acid; the resulting solution was allowed to stand overnight at room temperature. Then the solution was heated at 60°C for 15 min., and the reaction product was extracted with ether. The ether solution was washed succesively with a solution of sodium bisulfite, water, a solution of sodium carbonate and water. When the ether was distilled off, 162 mg. of the reaction product remained. To a solution of this material in 5 ml. of ethanol was added the reagent prepared from 0.2 g. of 2,4-dinitrophenylhydrazine, 1 ml. of sulfuric acid, 1.5 ml. of water and 5 ml. of ethanol. The mixture was heated at 60°C for 10 min. and then allowed to stand for 24 hr. The precipitates of crude DNPH formed were filtered. Yield, 152 mg. These were purified by chromatography on silica gel and elution with hexane and hexane-ether. The material obtained from the main eluate fraction was purified further by repeated recrystallizations from hexane to give, eventually, 35 mg. of yellow needles of m. p. 84.5~85.5°C.

Found: C, 57.13; H, 6.91; N, 14.83. Calcd. for $C_{18}H_{26}N_4O_5$: C, 57.13; H, 6.93; N, 14.81%.

The Oxidative Cleavage of the Saturated Alcohol with Potassium Permanganate. — To a solution of 90 mg. of the saturated alcohol in 30 ml. of acetone an excess of potassium permanganate in small portions was added, and the mixture was refluxed for 7 hr. The reaction product was treated in the usual way, and 46 mg. of an acidic substance was separated. This was converted to the methyl ester, and the latter was analyzed by gas-liquid chromatography (temperature: 160°C). Methyl pelargonate, together with a small amount of methyl caprylate and oenanthate, was identified.

The Preparation of *n*-Dodecane by the Reduction of the Reaction Product of the Saturated Alcohol and Hydrogen Iodide. — Six grams of hydrogen iodide (d=1.7) were added to a solution of 205 mg. of the saturated alcohol in 2 ml. of glacial acetic acid, and the mixture was refluxed for 1 hr. The reaction product was extracted with ether, and the ether solution was washed successively with a solution of sulfurous acid, a solution of sodium bicarbonate, and water. When the ether was distilled off, there remained 380 mg. of the reaction product, which was regarded as alkyl iodide by its infrared spectrum.[*4] To a solution of 160 mg. of this iodide in 35 ml. of ether, which had been dried over metallic sodium, 200 mg. of lithium aluminum hydride were added and the solution was refluxed for 2 hr. The reaction product was separated in the usual way. Yield, 63 mg. *n*-Dodecane was identified by gas-liquid chromatography (temperature: 100°C).

The Ozonolysis of the Debromination Product of the Alkyl Bromide Derived from the Saturated Alcohol.—To a solution of 190 mg. of the saturated alcohol in 2 ml. of glacial acetic acid 10 g. of hydrogen bromide (d=1.49) were added, and the mixture was refluxed for 5 hr. The reaction product was separated in the usual way. Yield, 267 mg. This was considered to be alkyl bromide from its infrared spectrum.[*4] The bromide was dissolved in 15 ml. of glacial acetic acid, and 750 mg. of zinc powder were added to the solution. After this solution had been refluxed for 2 hr., the debromination product was obtained by extraction with ether. The debromination product (130 mg.) in 10 ml. of methylene chloride was ozonized at −15°C. The ozonide was decomposed by adding 5 ml. of a 10% solution of potassium hydroxide and 1 ml. of 30% hydrogen peroxide, after which the mixture was gradually heated to 85°C while passing a current of nitrogen through it. The alkaline solution was then acidified with diluted sulfuric acid and distilled. The distillate, after neutralization with a N/10 solution of potassium hydroxide, was evaporated, and the residue was taken up with ethanol. The ethanol solution was concentrated to a volume of 2~3 ml. Benzyl bromide in an amount equivalent to the alkali required for the neutralization of the distillate was added to the concentrated ethanol solution, and the mixture was refluxed for 25 min. The benzyl esters thus obtained were extracted with ether. Yield, 63 mg. These were analyzed by gas-liquid chromatography (temperature: 200°C); benzyl propionate and pelargonate were identified as the main components.

Discussion

Since cholesterol and batyl alcohol are the main components of the crystalline solid which was obtained by recrystallizations of the total nonsaponifiable matter from methanol and acetone, and since batyl and selachyl acetates are found as main components of the higher-boiling acetate fractions (Table I) of the liquid nonsaponifiable matter, cholesterol, batyl

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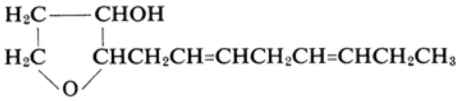
[*4] The absorption bands associated with the hydroxyl and ether groups in the original unsaturated alcohol were absent in the spectrum of this substance.

alcohol and selachyl alcohol are considered to be major components of the total nonsaponifiable matter.

An acetate fraction (b. p. 130∼135°C/2 mmHg) obtained by the fractional distillation of the acetate of liquid nonsaponifiable matter accorded with Tsujimoto's kanyl acetate in its properties, but this fraction was found to be a heterogeneous mixture by gas-liquid chromatography. Of the two eluate fractions (A and B) obtained by column chromatography of this fraction on silica gel, fraction A consisted of a uniform substance, while fraction B was found to be a mixture of different components. Hence, kanyl alcohol is not a uniform substance but a mixture which contains a peculiar alcohol as a main component, namely, the alcohol of fraction A. Since fraction A was readily separated by column chromatography, it appears to be of a series quite different from the other component acetates.

It may be seen from Fig. 2 that the infrared spectra of fraction A and the free alcohol obtained therefrom show an absorption band at 1075 cm^{-1} which may be assigned to the C-O stretching vibration of aliphatic ethers or five- or six-membered cyclic ethers,[5] while the infrared spectrum of the free alcohol shows an absorption band at 1105 cm^{-1} which may be assigned to the C-O stretching vibration of secondary alcohols. Since the formula of the saturated alcohol obtained by the hydrogenation of fraction A is demonstrated as $C_{12}H_{24}O_2$ by analyses of its phenyl urethan and of the DNPH obtained from its oxidation product (ketone), the formula of the unsaturated alcohol should be $C_{12}H_{20}O_2$, since it has two double bonds, as is seen from its iodine value of 241.4. The proportion of carbon and hydrogen atoms indicated that there should be a cyclic group in its molecule.

Fraction A has an alkali-conjugatable diene $=CHCH_2CH=$, and the ozonolysis of fraction A gives propionaldehyde as a cleavage product. Accordingly, fraction A should have the group $CH_3CH_2CH{=}CHCH_2CH{=}CH-$. Further, the oxidative scission of the saturated alcohol with potassium permanganate yields pelargonic acid as a main cleavage product. Hence, the saturated alcohol should have a straight chain of 9 carbon atoms; $CH_3(CH_2)_7CH<$. This fact, together with the fact that the reduction of the alkyl iodide derived from the saturated alcohol with lithium aluminum hydride yields *n*-dodecane, leads to the conclusion that the unsaturated alcohol is α-octa-2, 5-dienyl-β-hydroxytetrahydrofuran:



This structure is also consistent with the fact that the ozonolysis of the debromination product of the alkyl bromide derived from the saturated alcohol yields pelargonic and propionic acids.

Department of Applied Chemistry
Faculty of Engineering
Nagoya University
Chikusa-ku, Nagoya

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[5] L. J. Bellamy, "The Infrared spectra of Complex Molecules," Methuen & Co., Ltd., London (1958), pp. 116, 119.